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Menadione effect on isolates of bone cultures in patients with chronic osteomyelitis culture-negative

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Correct treatment of chronic osteomyelitis depends on proper identification of the bone-infecting microorganism, but it is difficult identify the specific etiology in previously treated patients and in those with implants. Small colony variants auxotrophyc for menadione had been related with false-negative results in culture of patient with chronic osteomyelitis, but menadione supplementation can increase bone culture performance. The purpose was to evaluate the effect of menadione supplementation on isolates in bone cultures, in a cohort of patients with osteomyelitis, Medellín-Colombia. We performed a study of a retrospective cohort with 40 adult patients with culture-negative and chronic osteomyelitis, supplemented with 3 doses of menadione. Effect was defined as the proportion of positive bone cultures after treatment administration. The comparison of the effect with clinical variables was made with Chi-square, Fisher and Mann-Whitney U test in SPSS 29.0. Microbiological isolates from bone culture ranged from 0% (pre-treatment) to 62.5% (post-treatment), mainly S. aureus sensitive to methicillin, coagulase-negative Staphylococcus, E. coli and Enterobacter spp. This effect did not present statistical differences according to the clinical characteristics or comorbidities of the patients. We concluded that in patients with chronic osteomyelitis and negative bone cultures, menadione supplementation produces a high proportion of isolates and identification of the etiological agent, which favors correct treatment and reduces readmissions, complications, and resistance to antibiotics.

Keywords: Menadione, Vitamin K, Ostemyelitis, Bone, Culture, Small Colony Variants.

INTRODUCTION

The advances in diagnosis, differentiated approaches and treatments of osteomyelitis have made it possible to impact morbidity and mortality from this cause[1]. However, antibiotic therapy for this disease continues to represent a challenge, as evidenced in some studies that report a high rate of resistance to common antibiotics (e.g. 83% of S. aureus isolates are resistant to oxacillin or methicillin), long duration of bone infection (3 months to 30 years) and high number of operations[2]. Recent reviews of the literature indicate that one of the main mechanisms to generate chronic osteomyelitis is drug-resistant bacteria, of which cellular biofilm is the most important cause due to its protective effect for the microorganism; besides, biofilm enhances the resistance to antibiotics, obstruct bacterial clearance and increase the risk of recurrent infections[3].

Although biofilms may limit the ability to recover bacteria using standard culture techniques, there are other mechanisms involved such as Small Colony Variants (SCVs): a subpopulation of bacteria within biofilms that grow very slowly and in vivo-culture results in a subgroup smaller than standard colonies[4,5]. SCVs has several relevant consequences such as protect bacteria from host defenses, increases resistance to antibiotics, and their slow growth makes them difficult to culture[6].

SCVs was first linked to persistent and recurring infections by Proctor et al.[7] when reported SCVs of S. aureus from 5 patients with unusual antibiotic resistant infections. The report was the first model that proposed a relationship between SCVs and the clinical pattern of persistent, relapsing infection[8]. Subsequent studies have confirmed that S. aureus SCVs cause various forms of infection including cystic fibrosis, foreign-body related infections and osteomyelitis[9-11]. In orthopedics several authors suggest that SCVs can cause recurrent infections in bone and implants; failure to account for their unique properties, particularly their reduced growth rate, may yield a false-negative results of cultures[12].

SCVs seems to be a natural survival mechanism for many bacterial species[13]. Some SCVs can be distinguished by supplementation with a particular substrate in a chemically defined medium[14].

Supplementation either causes reversion of the growth to the normal phenotype or enhances the growth of the SCV surrounding a disc, which was impregnated with this particular substrate[15]. By this method, menadione-, hemin-, thymidine-, and CO2-dependent SCVs have been determined, and the menadione auxotrophy can be restored to wild-type colony morphology and growth characteristics, by menadione (vitamin K3) supplementation[16,17].

Despite this background, in the world scientific literature there are few studies on SCVs, the most focused in S. aureus, and there are no researches that relate SCVs, chronic osteomyelitis and the effect of menadione supplementation in patient cultures[14,18-20]. Therefore, the objective of this investigation was to evaluate the effect of menadione supplementation on isolates in bone cultures, in a cohort of patients with osteomyelitis, Medellín-Colombia.

MATERIAL AND METHODS

Setting and design

Study of a retrospective cohort of 40 patients. The study was conducted at Clinica Las Vegas, a 200-bed, third level university-based clinic located in Medellín, Colombia. The clinic is a referral center for patients from four departments known as the coffee-growing region and includes the departments of Antioquia, Caldas, Quindío and Risaralda (Figure 1). Medical charts of patients with diagnosis of chronic osteomyelitis recorded in the Infectious Diseases Section, were reviewed by a medical. During the time analyzed (2014-2022), there were no changes in clinical, diagnostic or surgical practices (it was ensured that the procedures applied to all the patients included in this study were the same during the 9 years reviewed). The diagnosis of chronic osteomyelitis was defined as: clinical pattern evolved over months or years and characterized by tissue inflammation, presence of pus, sinus tract, fistulae, or presence of dead bone (sequestrate), demonstrated by plain film, computed tomography or magnetic resonance imaging[21].

Eligibility criteria

Inclusion criteria were patients over 18 years-old with a clinical and radiologic diagnosis of chronic osteomyelitis, who had a first negative aerobic and anaerobic bacterial culture from the compromised bone. Based on inclusion criteria 1, the information of 1006 patients were reviewed, of which 775 who had positive cultures were eliminated. Subsequently, we excluded any of the following non-bone specimens directly related to the infected bone (n=191): pus aspirated from surrounding soft tissues, soft tissues, surgical wounds, drainage from orifices left by orthopedic pins, and drainage from sinus tracts. Only four operative bone specimens were acceptable: bone biopsy, sequestrum, bone marrow, and aspirated subperiostic pus. It was also required that bone specimens had to be taken during surgery, and a clear note by the surgeon must establish if the incision was

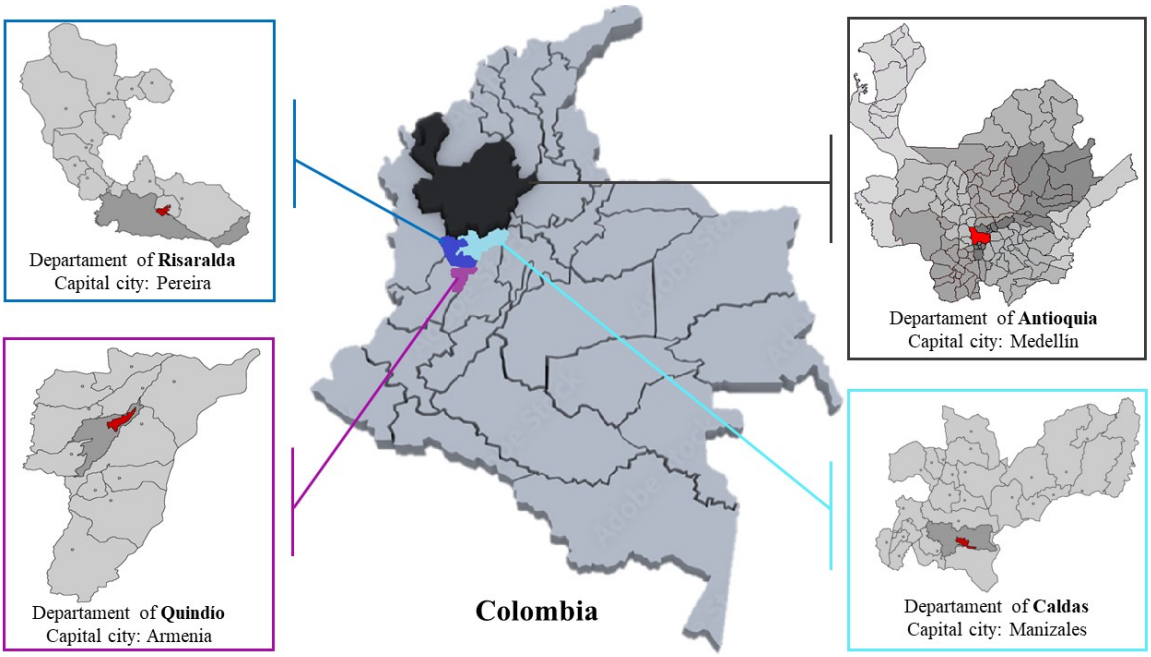

Fig. 1 — Site of study.

made through intact skin in opposition to infected soft tissues or sinus tracts. After the application of the exclusion criteria, the retrospective cohort was defined with 40 patients who received the treatment. The sample size met the following parameters for comparison of paired proportions: initial proportion of 1% (0% was not taken, which would be correct in this study, because it would not generate any statistical estimate), proportion 2 of 30% (the minimum level of effect expected after supplementation with menadione), confidence of 95%, and statistical power of 95% for a total of 40 patients with two measurements each.

Procedures

After a first negative aerobic and anaerobic bacterial bone culture, each patient received 10 mg per day orally of Menadione for 3 days, and after de 4th day, the surgical bone culture was repeated again. Microbiologic identification and susceptibility patterns of organisms grown in aerobic and anaerobic atmosphere, was performed using Vitek-2 Systems; and Rapid ID32A, from BioMerieux. Routine and standard laboratory techniques for transportation and culture of aerobic and anaerobic microorganisms were followed, according to CLSI (Clinical & Laboratory Standards Institute) standard procedures.

Gathering information and Bias control

The medical evaluation was carried out by specialists in infectious diseases, recording the symptoms upon admission, comorbidities, diagnoses and previous treatments, complications, hematic profile, hospital stay, culture results before and after supplying menadione and identified microorganisms.

To avoid selection biases, the inclusion of the study subjects was carried out with the application of clinical criteria by the infectologist; in addition, several actions were implemented to mitigate this possible bias, such as standardizing the patient selection process, using unbiased tools and software (in cases where possible, digital medical history was applied), blind and independent collection of clinical data, participation of several health professionals in the selection process (physician, infectious disease specialist, orthopedist).. To avoid information biases, laboratory tests with high validity and internal and external quality control were applied; the information was collected by the medical team that provides routine care for this type of patient and the completion of the clinical data for this study was standardized. The information was collected in a flat Excel file, in an anonymized database by the clinic's medical team; in the migration of data from the primary source to the database for analysis, a random check of all data recorded on 10% of the patients was performed, and double entry of the variables was also performed.

Statistical analysis

Analyzes were performed with absolute (n) and relative (%) frequencies. No control group was defined, but the measure of effect was defined as the number of patients who had a positive culture after treatment (compared with their initial condition, which was to have a negative culture). The comparison of the result of the culture (categorized as positive or negative) after the administration of menadione was made with all the study variables using Chi-square for the nominal ones, Fisher when the expected frequency was less than 5 in some subgroup, and Mann-Whitney U test for continuous data, since the data did not meet the assumption of normality according to the Shapiro-Wilk test. SPSS 27.0 was used for the analyses, taking as significant p values less than 0.05.

Ethical considerations

All participants signed the informed consent. The project complied with the guidelines of the Declaration of Helsinki and Resolution 8430 of the Colombian Ministry of Health of 1993. The project was endorsed by the ethical and scientific committee of the clinic minute CEI-146.

RESULTS

In 9 years, we found 40 patients with diagnosis of chronic osteomyelitis and had a first negative aerobic and anaerobic bacterial bone culture. From them, 19 (45.2%) were women and 23 (54.8%) men, with a median age of 55.5 years (IR = 37.5-71.2) and range 16-84 years. The 52.5% (n=21) residents of Medellin, the remaining percentage coming from other cities; the majority (87.5%) affiliated with the contributory health regime and from the middle social class (80%).

The main symptoms on admission were discharge (64.3%), edema (64.3%), erythema (61.9%), pain (50.0%), ad fever (26.2%). The main comorbidity was hypertension (35.7), immunosuppression (19.1%), mellitus diabetes (16.7%), obesity (11.9%), and hypothyroidism (11.9%). The 42.6% had a history of chronic osteomyelitis with previous positive cultures, and 83.3% had previous use of antibiotics (Table I). In general, these were patients without many comorbidities, hemodynamically stable,

without bacteremia, and without significant clinical complications.

In the cases of immunosuppression, 7 corresponded to rheumatoid arthritis and one to psoriasis. Septic arthritis occurred in the foot (n=2), ankle (n=1) and knee (n=1). In allergy to antibiotics, two cases were to vancomycin, and one for each of the following cases: Penicillin, Clindamycin, Cephalexin, Sulfas, and Rifampicin. Previous isolations were obtained in ankle (n=2), tibia (n=2), fibula (n=3), elbow (n=1), femur (n=1), ischium (n=1), knee (n=1), finger (n=1), hip (n=1), shoulder (n=1).

Location of the osteosynthesis material: hip (n=7), knee (n=7), ankle (n=2), shoulder (n=2), hand (n=2), fibula (n=2), tibia (n=2), lumbar spine (n=2), femur (n=1), humerus (n=1), elbow (n=1). Location of previous osteomyelitis: knee (n=5), tibia and fibula (n=3), hip (n=2), foot (n=3), fibula (n=2), femur (n=2), ankle (n=2), elbow (n=1), hand finger (n=1), humerus (n=1), ischium (n=1).

The median hospital stay was 22 days. Within the laboratory studies, it stands out that in the blood profile the median hemoglobin value was low and the erythrocyte sedimentation rate (ESR) was high; the median number of days of treatment after the menadione cycle was 30 days (Table II).

After administering the menadione and repeating the bone culture, a positive microbiological isolation was achieved in 62.5% of the patients (two agents were isolated in seven patients); among these, 32% were methicillin-sensitive S. aureus, 32% coagulase-negative Staphylococcus (1 methicillin-sensitive S. epidemidis, 4 methicillin-resistant S. epidermidis, and 3 S. lugdunensis), E. coli, and Enterobacter spp. 12.0% (E. aerogenes with one case and E. cloacae with two cases), and in the category of others, one case of

Table I. — Description of the clinical findings in the study group.

	n	%
Symptoms on admission		
Fistula or discharge	27	64.3
Edema	27	64.3
Erythema	26	61.9
Pain	21	50.0
Fever	11	26.2
Bone exposure	3	7.1
Exposure of osteosynthesis material	3	7.1
Comorbidities		
Arterial hypertension	15	35.7
Immunosuppression	8	19.1
Mellitus diabetes	7	16.7
Obesity	5	11.9
Hypothyroidism	5	11.9
Heart failure	3	7.1
Chronic kidney disease	2	4.8
Other conditions		
Smoking	7	16.7
Allergy to antibiotics	7	16.7
Presence of osteosynthesis material	29	69.0
Previous diagnosis and treatment		
Previous diagnosis of chronic osteomyelitis	23	54.8
Surgical site infection (SSI)/cellulitis	19	45.2
Septic arthritis	4	9.6
Previous isolations	18	42.6
Prior antibiotic treatment	35	83.3
Single antibiotic treatment	14	40.0
Treatment with 2 or more antibiotics	21	60.0

Table II. — Description of clinical and paraclinical characteristics of the study.

Variable	Median (IR)	Range
Days of hospital stay	22 (15-43)	8-71
Hemoglobin (mg/dl)	11 (10-14)	6-18
Leukocytes (in thousands)	8.5 (7.2-11.4)	4.1-7.2
Platelets (in thousands)	247 (148-311)	53-894
Erythrocyte sedimentation rate	31 (12-45)	2-120
C-Reactive Protein	8 (1-20)	0-361
Days of antibiotic treatment (post menadione)	30 (30-42)	28-42

each of the following agents was found: Streptococcus gordonii, Acinetobacter baumannii, Corynebacterium spp., Pseudomonas fluorescens, Serratia marcescens and Proteus mirabilis (Figure 2). In addition, safety was found in all patients with oral menadione, since there were no complications or adverse effects from its consumption.

The median number of days with antibiotic treatment after the intervention with menadione was 30 (IR 30-42). In 42.5% (n=17) the osteosynthesis material was removed. Most of the patients with positive cultures after the use of menadione had the presence of osteosynthesis material, only 5 patients did not have osteosynthesis material (it had been previously withdrawn), but they had a history of chronic osteomyelitis, and recent use of antibiotics.

After administering the menadione cycle, some subgroups presented a higher proportion of positive cultures, but without statistically significant differences: patients with fistula or discharge (72%), with fever (82%) and with immunosuppression (75%). In these analyses, 8 of 19 comparisons with clinical variables presented satisfactory statistical power; the comparisons with statistical power <80 corresponded to the cases in which the proportion of post-treatment positive isolates was very similar between the groups analyzed (differences were not clinically relevant) (Table III).

In general, no clinical characteristic of the patient affected the positivity of the culture after the intervention with menadione, which is equivalent to saying that this intervention is equally effective in all subgroups of patients analyzed (it is independent of the clinical conditions evaluated). The type of germ isolated prior to supplementation with menadione did not condition its use; there was no relationship with the affected site or compromised joint, and effectiveness was demonstrated even in the spine, knee, hip, shoulder, and long bones; sex, age and type of osteosynthesis material were variables that did not influence the results.

DISCUSSION

An increasingly common problem in the attention of patients with chronic osteomyelitis is relapses or exacerbations of chronic infections with negative cultures, which in the presence of osteosynthesis material or previous use of antibiotics may be related

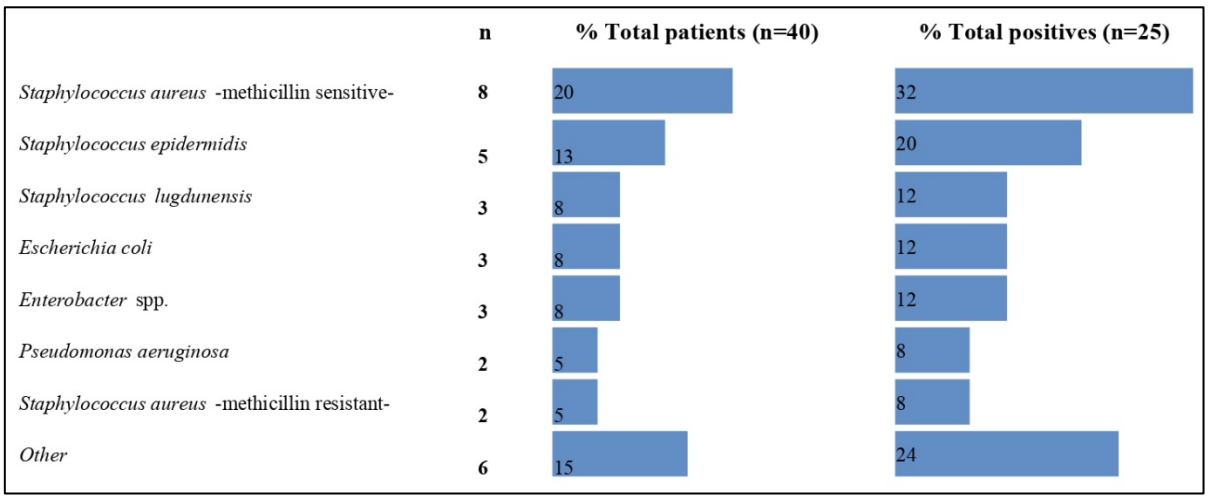

Fig. 2 — Isolates after the intervention with menadione.

Table III. — Frequency of positive isolates after the intervention with menadione, according to the clinical conditions of the patients.

Variable	Clinical feature		[a] p Chi² [b] p Fisher	1-β
	Present	Absent		
	% (n) positive isolation*			
Symptoms on admission				
Fistula or discharge	72.0 (18)	46.7 (7)	0.109 [a]	0.95**
Edema	65.4 (17)	57.1 (8)	0.608 [a]	0.28
Erythema	68.0 (17)	53.3 (8)	0.875 [a]	0.60
Pain	60.0 (12)	65.0 (13)	0.744 [a]	0.16
Fever	81.8 (9)	55.2 (16)	0.120 [a]	0.98**
Bone exposure	66.7 (2)	62.2 (23)	0.688 [b]	0.15
Osteosynthesis material exhibition	33.3 (1)	63.0 (17)	0.523 [b]	0.99**
Comorbidities				
Arterial hypertension	50.0 (7)	69.2 (18)	0.231 [a]	0.81**
Immunosuppression	75.0 (6)	25.0 (2)	0.686 [b]	1.00**
Mellitus diabetes	66.7 (4)	61.8 (21)	0.600 [b]	0.16
Obesity	60.0 (3)	62.9 (22)	1.000 [b]	0.09
Heart failure	66.7 (2)	62.2 (23)	1.000 [b]	0.15
Chronic kidney disease	100.0 (1)	61.5 (24)	0.625 [b]	1.00**
Other conditions				
Smoking	40.0 (2)	65.7 (23)	0.345 [b]	0.95**
Osteosynthesis material	59.3 (16)	69.2 (9)	0.542 [a]	0.80**
Previous diagnosis and treatment				
Previous diagnosis of chronic osteomyelitis	63.3 (14)	61.1 (11)	0.870 [a]	0.08
ISO/Cellulite	61.1 (11)	63.6 (14)	0.870 [a]	0.09
Septic arthritis	50.0 (2)	63.9 (23)	0.622 [b]	0.55
Prior antibiotic treatment	60.6 (20)	71.4 (5)	0.691 [b]	0.42

* It represents the effect of supplementation with menadione (% positive isolates) within those who presented and within those who did not register each one of the clinical events indicated in each row.
*Satisfactory statistical power (≥0.80).

to the presence of SCVs[9,22,23]. These SCVs have mutations and metabolic alterations, which facilitate their persistence in different bone tissues[13,14,24]. One of the main metabolic alterations described is Menadione axotropism[25], which hinders its proper culture and identification in conventional laboratories, generating false negatives in cultures related to orthopedic infections[8,11,26]. One of the most used measures to reverse this auxotropism is Menadione supplementation, so that, by repeating bone cultures, performance of cultures can be increased and the causal agent can be identified[10,12,27], but unfortunately clinical experience published in this regard is scarce, which limits the discussion of our findings.

In the present study 40 patients with chronic osteomyelitis were analyzed, who had a negative initial culture, both for aerobic and anaerobic, after which they were supplemented with 3 doses of Menadione, and cultured again, obtaining an increase in culture performance with a positivity of 62.5%, which constitutes a valuable finding to establish a precise etiology of these infections, since it avoids the excessive and irrational use of antibiotics empirically, thus also limiting the selection of different strains resistant to antibiotics[6,8].

When we analyze the different clinical factors that could be associated with the results, none of them had a statistical association with the higher performance in the cultures after supplementation with Menadione; however, it should be highlighted that 83.3% of the patients had previous consumption of antibiotics, and 69% had the presence of implants (osteosynthesis material), which may facilitate the axotropism described in this type of bacteria, and therefore this variables should be taken into account in medical chart when dealing with orthopedic infections with these particular characteristics[28].

Although we know that any bacterium has the metabolic capacity to have auxotropism by Menadione[13,29], it is clearly described that Staphylococcus spp. are the most frequently involved[26-28,30,31], a fact that is also corroborated. in this study, where Staphylococcus spp. represented 64% of the isolates recovered after supplementation. However, this investigation shows this possible mechanism in other less frequent etiological agents

in chronic osteomyelitis (E. coli, Enterobacter spp., S. gordonii, Acinetobacter baumannii, Corynebacterium spp., Pseudomonas fluorescens, Serratia marcescens and Proteus mirabilis), which it constitutes a novel finding, since the predominant evidence in this field is in S. aureus[14,15,17-20,24,25].

This study presented several limitations for the extrapolation of the results to other groups: the high exigency of the eligibility criteria with clinical evaluations carried out by medical specialists that only apply to the institutionalized population of hospitals with a high level of care complexity, and geographical, economic and others barriers to access in health related to the type of insurance and health care in Colombia that prevent adequate coverage of specialized health services for this type of patient, which was reflected in the size of the retrospective cohort.

Although traditional studies about effectiveness are conducted with two or more groups (treatment vs. control), in this research it was not possible to apply this design for the following reasons: i) because the specificity of the clinical condition evaluated paired analyses were required, and each subject must be his/her own control; ii) the clinical condition is rarely evaluated and diagnosed in Colombia as demonstrated in the description of the population (in almost a decade of records in the main clinic of the city only 1,006 patients were found, and only 40 met inclusion criteria), and iii) having two groups to randomize the intervention would reduce the statistical power of the comparison because there would not be 40 data per group but 20 data per group. However, this design did not affect the quality of the evidence given that the most rigorous design possible for this type of clinical situation was applied, taking each subject as his/her own control. If an independent control group had been designed without treatment, the effect measure for comparison would be the same, since the central outcome of the study (post-treatment positivity) would be compared against a group with result zero, considering the eligibility criteria required by this clinical condition, which would imply the inclusion of a patient with a negative culture and without treatment.

It was not possible to conduct a multicenter study in key cities to cover the entire country for three reasons: i) similar clinical management of the patients could not be ensured in the several centers consulted, ii) in several clinics in other cities, menadione was not supplied to those who met the eligibility criteria, and iii) the bioethics committee of other hospitals consulted required preliminary evidence on the effectiveness in Colombian patients, which was not available at the start of the research project (this study represents seminal evidence on this topic with Colombian patients). Subsequent research can be conducted to generalize this evidence to Colombian patients with this clinical condition.

It is necessary further research into the mechanisms of SCV formation and persistence in chronic osteomyelitis would provide deeper insights and potentially identify new therapeutic targets.

CONCLUSION

In patients with chronic osteomyelitis and negative bone cultures, menadione supplementation increases to 62.5% the probability of isolating and identifying the precise etiological agent, using conventional microbiological cultures, independent of the compromised bone, comorbidities and other the patient's clinical characteristics. It has a positive impact, since a treatment directed at the isolated germ can be established, favoring the adoption of appropriate behaviors, reducing the development of resistance, readmissions and future complications.

Data Sharing Statement: All relevant data are included in the manuscript.

Ethics Approval and Informed Consent: This study was conducted in accordance with the Declaration of Helsinki and its revisions, and approved by the ethical committee of FAI. Written informed consent was obtained from all patients who participated in this study.

Author Contributions: All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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