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Antibacterial Activity of Antibiotic-Impregnated Bone Cement Based Coatings Against Microorganisms with Different Antibiotic Resistance Levels

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Abstract

Purpose — to evaluate the presence and duration of antibiotic activity of antibiotic-impregnated bone cement based coatings samples against antibiotic-sensitive and antibiotic-resistant microorganisms. ***Materials and Methods.*** Bone cement based coatings impregnated with antibiotics (gentamycin, vancomycin, colistin, meropenem, fosfomycin) are formed on titanium (Ti) plates. A plate rinse was carried out; antibiotic concentrations in the rinsed solutions were estimated by a serial broth microdilution method. Antibacterial activity of the control and rinsed samples against the antibiotic-sensitive and multiple-antibiotic-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains was estimated by a bilayer agar method. ***Results.*** The meropenem and fosfomycin concentrations in the rinsed solutions obtained at a one-fold (16 μg/ml for both antibiotics) and two-fold treatment (2 μg/ml for meropenem and 8 μg/ml for fosfomycin) were sufficient to suppress the growth of the control strains. One-fold rinse of samples with colistin eliminated their antibacterial activity completely. The marked activity of the samples with meropenem and fosfomycin persisted against the antibiotic-sensitive *P. aeruginosa* ATCC 27853 strain after 2 rinse cycles; single-rinsed samples with fosfomycin also maintained the activity against the extensively antibiotic-resistant *P. aeruginosa* BP-150 strain. Vancomycin-containing samples possessed the sufficient antibacterial activity against both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *S. aureus* strains; two-fold rinse of the samples eliminated their bactericidal properties. ***Conclusion.*** Bone cement based coatings impregnated with fosfomycin and meropenem possess the most marked and long-lasting antibacterial activity, manifested mainly against the antibiotic-sensitive strains.

Keywords: bone cement, meropenem, fosfomycin, colistin, local antibiotic therapy.

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Background

Gram-positive bacteria (*S. aureus* and coagulase-negative staphylococcs, *Enterococcus* spp., *Streptococcus* spp.) are the main etiological agents of bons and joint infections, while the amount of gram-negative bacteria accounts for no more than 10–22% of all cases, including 2–7% for *P. aeruginosa* [1, 2]. Gram-negative microorganisms are often detected in open fractures, chronic osteomyelitis and periprosthetic infections [3]. In cases of periprosthetic implant-associated infections, the proportion of *P. aeruginosa* in etiology may increase up to 20%. The constant increase of the etiological role of *P. aeruginosa* with multiple antibiotic resistance is noteworthy [4]. Cases of implant-associated infections and post-traumatic osteomyelitis caused by carbapenemase-producin, antibiotic-resistant (MDR and_XDR) *P. aeruginosa* strains have been

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documented [5, 6]. Clinical outcomes of infections caused by *P. aeruginosa* are significantly less favorable than outcomes of infections caused by staphylococci [7].

Systemic antibacterial therapy of bone and joint infections caused by gram-negative microorganisms is often ineffective. This may be due to the multidrug resistant bacteria, the heterogenecity of the bacterial population and the transition of a part of microbial cells to metabolically inactive forms, as well as the formation of microbial biofilms on the bone surface or on the surfaces of incorporated implants [6]. The main method of local antimicrobial therapy for bone infections is the use of polymethylmethacrylate (PMMA)-based bone cement impregnated with antibiotics. Several types of bone cements containing antibiotics (gentamicin, tobramycin, vancomycin) are widely used.

Ready-made bone cements contain low antibiotic concentrations and are intended to prevent infections. With the spread of antibiotic resistance in bacterial populations, aminoglycosides are gradually losing their significance, and the antibacterial spectrum of vancomycin does not cover gram-negative microorganisms. Therefore, it is necessary to choose other antibiotics that are effective, including against numerous multidrug and extensively drug-resistant gram-negative pathogens. In addition, the antibiotic should have thermal stability, a wide range of bactericidal activity in low concentrations, as well as the ability to elute from PMMA for a long time and maintain sufficient local inhibitory concentrations that prevent the proliferation of bacteria and the formation of microbial biofilms [8]. In practice, various antibiotics are widely used in bone cement: aminoglycosides, glycopeptides, cephalosporins, fluoroquinolones, colistin, linezolid, daptomycin [9].

Techniques for applying a layer of PMMA with an antibiotic on the surface of the intramedullary nails are proposed for the treatment of infected nonunions and osteomyelitis. The antibacterial layer on the surface of an intramedullary fixation device is prepared during surgery [10, 11]. In the available literature there is no evidence about the mechanisms and kinetics of release of antibiotics from PMMA-based coatings applied on the surface of medical implants. The possibility of using polymyxins, carbapenems, fosfomycin (i.e. antibiotics that remain active against various antibiotic-resistant gram-negative pathogens) in bone cement requires study.

The purpose of the study is to evaluate the presence and duration of antibacterial activity of antibiotic-impregnated bone cement based coating samples against antibiotic-sensitive and antibiotic-resistant microorganisms.

Materials and methods

Under aseptic conditions, the appropriate portions of pure antibiotic substances (vancomycin, colistin, meropenem, fosfomycin) were weighed. They were incorporated in 10 g of dry powdered bone cement (Subiton Gun, Laboratorios SL S.A., Argentina), then thoroughly mixed using a sterile spatula. 5 ml of monomer were added to the mixture, mixed and applied by a continuous uniform layer 0.5–1 mm thick onto BT-6 titanium plates 12.5×50×0.5 mm. Additionally, titanium plates with gentamicin-containing bone cement were prepared (Subiton Gun G, Laboratorios SL S.A., Argentina). the amount of antibiotics per 40 g of powdered bone cement was as follows: gentamicin — 0.5 g, vancomycin — 2 g, colistin — 0.24 g (3 000 000 IU), meropenem — 2 g, fosfomycin — 2 g.

After polymerization, titanium plates with applied bone cement were placed into sterile hermetically sealed polypropylene containers, labeled and divided into 3 groups. Each included 3 samples of the same type. Samples of group 1 were not rinsed and were used as control specimens. Samples of groups 2 and 3 were submerged in sterile isotonic NaCl solution (INaC*) with a volume of 100 ml and thermostatically incubated within 7 days in an ES-20 shaker-incubator (BioSan, Latvia) at 100 rpm and 35°C. Samples of group 3 were rinsed again in a fresh volume of INaCS for 7 days. Awaiting further study, the run-off solutions were stored frozen at -80°C. Antibiotic concentrations in the run-off solutions were determined by the method of serial microdilutions in Muüller-Hinton broth based on their ability to suppress the detectable growth of *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 with known Passport values of the minimum inhibitory concentrations (MICs) of these antibiotics.

Evaluation of the antibacterial activity of bone cement applied to titanium plates (for the

control and rinsed plates) was carried out using a bilayer agar method [12]. Plates were placed on the surface of Mueller-Hinton agar (Mueller Hinton II Agar, BD BBL, USA) using sterile tweezers in 90-mm polystyrene Petri dishes. Then 15 ml of melted and cooled to 45°C Mueller-Hinton agar was poured into the dishes forming a second layer (Fig. 1).

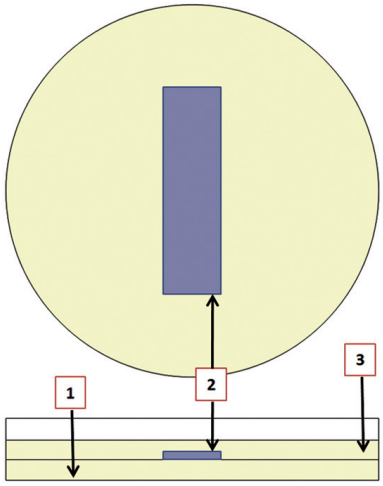

Fig. 1. Evaluation of the coating antibacterial activity by a bilayer agar method:
1 — 1st layer of nutrient medium;
2 — titanium plate coated with bone cement impregnated with antibiotic;
3 — 2nd layer of nutrient medium

The calculated thickness of the formed nutrient layer of the coated plate was 1.5–2 mm. The dishes were placed on a level horizontal surface until the medium completely set, then dried in a thermostat for 15 minutes.

Antibiotic sensitive microorganisms from the ATCC collection (*P.aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213) were used as test cultures for inoculation of Petri dishes with plates. Additionally, antibiotic-resistant strains of microorganisms isolated from patients with post-traumatic osteomyelitis were included in the study: *P. aeruginosa* P*-150 (resistane to most antibiotics, with the exception of polymyxins, VIM metallo-β-lactamase-produceg) and *S. aureus* 43431 (methicillin-resistant isolate — MRSA, resistane to oxacillin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, rifampicin).

Table 1 shows the MIC values of the antibiotics used in the bone cement composition for test cultures.

The Petri dishes were inoculated with bacterial suspensions (0.5 McFarland) using cotton swabs and incubated for 18 hours at 35°C. The presence of microorganisms and nature of their growth on the surface of Mueller-Hinton agar near the projection of plates with different coating compositions were evaluated.

Results

Table 2 shows the results of determining the antibiotic concentrations in the run-off solutions. Antibiotic-sensitive ATCC strains with the lowest MIC values were selected as indicator microorganisms. However, in some cases, the antibiotic concentrations did not inhibit the noticeable growth of test cultures, which was a limitation of the method. Thus, after the second rinsing of the gentamicin- and vancomycin-containing bone cement samples, the concentrations in the run-off solution were not sufficient to suppress the growth of test cultures.

Table 1

The MIC values of the antibiotics for test cultures of microorganisms

Microorganisms	MIC, μg/ml				
	gentamicin	meropenem	colistin	fosfomycin	vancomycin
E. coli ATCC 25922	0,5 (S)*	0,016–0,03 (S)	0,5–1 (S)	1 (S)	–
P. aeruginosa ATCC 27853	1 (S)	0,5 (S)	1–2 (S)	4 (S)	–
S. aureus ATCC 29213	0,25–0,5 (S)	–	–	1–2 (S)	1 (S)
P. aeruginosa PA-150	256 (R)	128 (R)	0,5 (S)	128 (R)	–
S. aureus 43431	64 (R)	–	–	–	0,5 (S)

*S — sensitive; R — resistant.

Table 2

Antibiotic concentrations in the run-off solutions

Antibiotic	Indicator microorganism to determine the antibiotic concentration	Antibiotic concentration, μg/ml	
		7 days	14 days
Gentamicin	*E. coli* ATCC 25922	4	<1
Meropenem	*E. coli* ATCC 25922	16	2
Colistin	*E. coli* ATCC 25922	<1	<1
Fosfomycin	*P. aeruginosa* ATCC 27853	16	8
Vancomycin	*S. aureus* ATCC 29213	8	<2

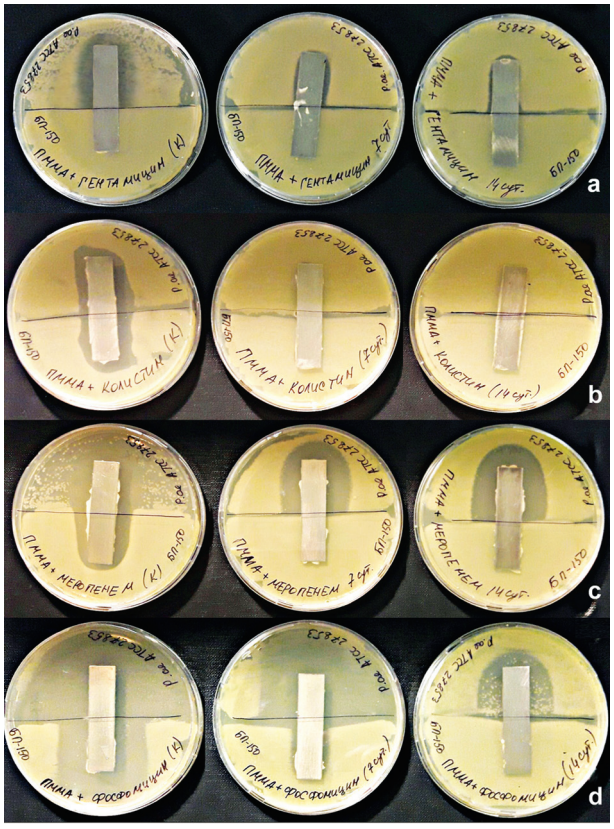

Fig. 2. Antibacterial activity of antibiotic-impregnated bone cement based coatings against *P. aeruginosa* ATCC 27853 (upper Petri dish sectors) and clinical isolate *P. aeruginosa* BP-150 (lower Petri dish sectors), a bilayer agar method:
a — gentamicin (0.5%);
b — colistin (0.6%);
c — meropenem (5%);
d — fosfomycin (5%)

The results of determining the antibiotic concentrations in the run-off solutions are consistent with the results of determining the antibacterial activity of the control and run-off samples (Fig. 2, 3). For control samples which weren't rinsed, in most cases antibacterial activity was revealed based on the absence of microorganism growth in the nutrient medium, both in the projection of coated plates and at different distances from them. The sizes of growth inhibition zones around the plates correlated with the MIC values of antibiotics of the studied strains. An exception was a gentamicin-containing bone cement which had antibacterial activity only against *P. aeruginosa* ATCC 27853 (MIC of gentamicin 1 μg/ml) and did not suppress the growth of an extensively antibiotic-resistant clinical isolate *P. aeruginosa* PA-150 (MIC of gentamicin >64 μm/ml).

Fig. 3. Antibacterial activity of bone cement based coating impregnated with 5% vancomycin against the antibiotic-resistant clinical isolate of *S. aureus* 43431 (MRSA), a bilayer agar method

Discussion

A detailed study of the kinetics of antibiotic release from bone cement in the form of spacers or beads in a fluid medium has been the subject of numerous earlier studies and therefore was not part of the objective of this work. It was shown that most antibiotic washout from bone

cement occurs during days 1–3 after implantation. Subsequently formed local concentrations fall below the MIC, which may induce the production of antibiotic resistance in microorganisms [13].

The concentrations of colistin in the run-off solutions (<1 μg/ml) obtained during this study were not sufficient to suppress the growth of *E. coli* ATCC 25922. This may be primarily due to the small amount of colistin compared to other antibiotics loaded in the bone cement (0.24 g per 40 g of cement). As shown in the research article by Gasparini et al., the elution of colistin from bone cement samples containing 0.6% (0.24 g per 40 g) of colistin sulfate ceased within 1 hour after being placed in the rinse solution of iNaCl, while barely 3.5% of the applied antibiotic was eluted. An increase of colistin to 2.4% in the content (0.96 g per 40 g of bone cement) prolonged its elution time to 7 days [14]. The inability of bone cement containing colistin (0.6%) and erythromycin (1.25%) to reduce the incidence of infectious complications in knee arthroplasty was shown in a randomized clinical study [15]. It is thought that the use of higher concentrations of colistin in the bone cement composition is associated with the risk of toxic effects. Thus, adding 2 g of colistin to 40 g of bone cement (5% concentration acceptable for most other antibiotics) would correspond to 400% of its maximum daily dose when administered intravenously [16, 17].

The most encouraging evidence was obtained for meropenem and fosfomycin. Their concentration in both rinse solutions (obtained 7 and 14 days after the elution started) significantly exceeded the MIC for antibiotic-sensitive strains. It was previously shown that bone cement containing 10% meropenem continues to release an antibiotic for 21 days, with 19% meropenem eluting into the solution [14]. The scientific work of V.A. Konev et al. shows a long-term (up to 28 days) antibacterial activity of bone cement containing 10% fosfomycin against antibiotic-sensitive *K. pneumoniae* and *S. aureus* strains [18].

A single rinse of the samples with colistin completely eliminated their antibacterial activity, which corresponds to published findings [14]. The pronounced activity of the samples against *P.aeruginosa* ATCC 27853 containing meropenem and fosfomycin was retained even after 2 cycles of rinsing. The samples containing fosfomycin also retained antibacterial activity against *P. aeruginosa* PA-150.

The *S. aureus* ATCC 29213 (MSSA) and *S. aureus* 43431 (MRSA) strains included in the study were not resistant to glycopeptides (MIC of vancomycin 1.0 μg/ml and 0.5 μg/ml, respectively), and similar results were shown when testing samples of bone cement impregnated with vancomycin. After a single 7-day rinse, the antibacterial activity of vancomycin-containing samples against *S.aureus* was retained. However, the size of growth inhibition zones around the plates was significantly reduced compared to the control samples (Fig. 3). A double 7-day rinse completely eliminated antibacterial activity.

In summary, bone cement impregnated with fosfomycin or meropenem coatings had the most pronounced and long-lasting antibacterial activity, which manifested itself mainly against antibiotic-sensitive strains. Effective use of colistin in coating composition is possible only with its increased concentration in the bone cement composition, which requires additional *in vitro* studies. Further research may be focused on the search for optimal antibiotic combinations in bone cement composition providing a more complete and prolonged elution into the surrounding tissues and having a synergistic effect on extensively antibiotic-resistant pathogens. Biodegradable polymers can be considered as alternative carriers for antibiotics capable of enabling a longer lasting local antibacterial effect against a wide range of antibiotic-resistant microorganisms. It seems appropriate to introduce into clinical practice standardized microbiological methods that allow evaluating the surface bactericidal activity of polymeric materials and coatings based on polymeric materials against clinical isolates of microorganisms obtained from specific patients.

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