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Prospects for using acetylsalicylic acid in combination with antibacterial drugs against the leading pathogens of osteomyelitis

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Abstract

Introduction Acetylsalicylic acid (ASA) is one of the promising candidates for enhancing the action of antibacterial drugs.

Purpose To evaluate *in vitro* the effect of aspirin in combination with antibacterial drugs on the growth of microbial cultures of clinically significant bacterial strains in osteomyelitis.

Materials and methods The object of the study is clinically significant bacterial strains K. *pneumoniae* (*n* = 12), P. *aeruginosa* (*n* = 12), S. *aureus* (*n* = 12), S. *epidermidis* (*n* = 12), isolated from wounds of patients with chronic osteomyelitis. The experiment was divided into four series: control group; group of ASA administration; group of antibiotic administration; group of ASA + antibiotic administration. Antibacterial effect was assessed by the absence of a bacterial growth zone around the discs.

Results There was no growth of microorganisms on Petri dishes when ASA (0.05 %) was incubated with gram-negative bacteria (K. *pneumoniae*, P. *aeruginosa*). In the case of S. *aureus* bacteria, the number of CFU was reduced compared to the control group. There was no growth on plates with S. *epidermidis*. When polymyxin was incubated with P. *aeruginosa*, slight bacterial growth was recorded on Petri dishes, while only individual colonies were observed with K. *pneumoniae*. When incubated together with polymyxin and aspirin (0.03 %), there was no growth of microorganisms on plates with K. *pneumoniae*, while slight growth was noted on plates with P. *aeruginosa*. Gentamicin suppressed the growth of S. *aureus* bacteria (an insignificant number of bacteria was noted on the plates). ASA (0.03 %) enhanced the bactericidal properties of gentamicin (there was no bacterial growth on the plates).

Discussion The absence of growth on plates with S. epidermidis bacteria under the influence of ASA, even without antibiotics, suggests that one of the mechanisms of its action may be the suppression of adhesion and biofilm formation. The presence of a pronounced effect of ASA on gram-negative bacteria (K. *pneumoniae*, P. *aeruginosa*) may be associated with the effect on the outer membrane of microbial cells, while aspirin had a lesser effect on gram-positive bacteria (S. *aureus*) and only reduced metabolic activity.

Conclusion Our study demonstrated that acetylsalicylic acid exhibits synergism with a number of antibiotics *in vitro* and significantly enhances their bactericidal activity against clinical isolates associated with osteomyelitis.

Keywords: acetylsalicylic acid, chronic osteomyelitis, S. *aureus*, S. *epidermidis*, K. *pneumoniae*, P. *aeruginosa*

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INTRODUCTION

Osteomyelitis is a severe infectious and inflammatory disease of bone tissue caused primarily by bacterial pathogens such as Staphylococcus *aureus*, Pseudomonas *aeruginosa*, and some other pathogens [1–4]. Despite current advances in antimicrobial therapy, osteomyelitis remains challenging to treat due to the ability of microbes to form biofilms, resistance to antibiotics, and chronic inflammation [5–7]. Therefore, a pressing area of research is the search for new therapeutic strategies that would include a combination of antibacterial drugs with adjuvant agents that can enhance their effectiveness.

Acetylsalicylic acid (ASA) is a promising candidate for enhancing the action of antibacterial drugs. In addition to its well-known anti-inflammatory and antiplatelet effects, aspirin has been shown to modulate antibiotic activity, inhibit bacterial biofilm formation, and influence the immune response [8–11]. In recent years, evidence has emerged of its synergistic effects with certain antibacterial drugs against resistant strains of microorganisms [12].

Purpose To evaluate *in vitro* the effect of aspirin in combination with antibacterial drugs on the growth of microbial cultures of clinically significant bacterial strains in osteomyelitis

MATERIAL AND METHODS

The object of the study is clinically significant bacterial strains K. pneumoniae (n = 12), P. *aeruginosa* (n = 12), S. *aureus* (n = 12), S. *epidermidis* (n = 12), isolated from wounds of patients with chronic osteomyelitis.

The analysis of biomaterials obtained from patients was performed in accordance with accepted international standards (UK SMI). Species identification of microbial cultures was performed using MALDI-TOF mass spectrometry. The susceptibility of bacteria to antimicrobial drugs (K. *pneumoniae* — to ciprofloxacin, cefepime, imipenem, meropenem; P. *aeruginosa* — to ciprofloxacin, cefepime, imipenem, meropenem, amikacin, piperacillin/tazobactam; S. *aureus* and S. *epidermidis* — to vancomycin, gentamicin, ciprofloxacin, erythromycin, cefoxitin), included in the formulary list of the Center, was determined according to the EUCAST criteria (European Committee on Antimicrobial Susceptibility Testing, Version 9.0, valid from 2019-01-01).

Inclusion criteria: clinical strains within the same taxonomic group, characterized by similar susceptibility to the tested antibacterial agents. Only methicillin-sensitive staphylococcal strains were included in the experiment.

Reference strains of bacteria belonging to four taxonomic groups were used as test cultures: Pseudomonas *aeruginosa* ATCC 27853, Klebsiella *pneumoniae* ATCC 700603, Staphylococcus *aureus* ATCC 29213, Staphylococcus *epidermidis* ATCC 14990.

The experiment tests were 4 series*и*:

— control series: suspensions of the studied cultures were prepared, containing 5×10^6 CFU/ml (colony forming units) of bacteria;

— second series (0.03 % ASA, 300 μg/ml): in the second part of the experiment, 1 ml of ASA was added to test tubes with cultures containing 5×10^6 CFU/ml of bacteria and incubated at room temperature for 30 min;

— third series: polymyxin (5 mg/ml) in the case of gram-negative bacteria and gentamicin (40 mg/ml) in the case of gram-positive bacteria were incubated at room temperature for 30 min together with suspensions containing 5×10^6 CFU/ml of microbial cells;

— fourth series: bacterial suspensions (5×10^6 CFU/ml) were incubated with a combination of ASA (0.05 %) + antibiotics (polymyxin or gentamicin) for 30 min.

Polymyxin is active against resistant gram-negative bacteria (P. *aeruginosa*, A. *baumannii*, K. *pneumoniae*, E. *coli*) and is the drug of choice for treating infections caused by carbapenem-resistant bacteria. Polymyxins act by disrupting the outer membrane of gram-negative bacteria (acting as a detergent). Gentamicin has high bactericidal activity against staphylococci.

To obtain a lawn, microbial suspensions in each experimental series were applied using a cotton swab to the surface of Mueller-Hinton agar. Antibiotic discs were placed on the surface and incubated for 24 hours in an incubator at 37 °C. Simultaneously, inoculations were made on Schaedler sheep blood agar (with hemin and vitamin K1) to monitor microbial cell growth. The results were assessed after 24 hours.

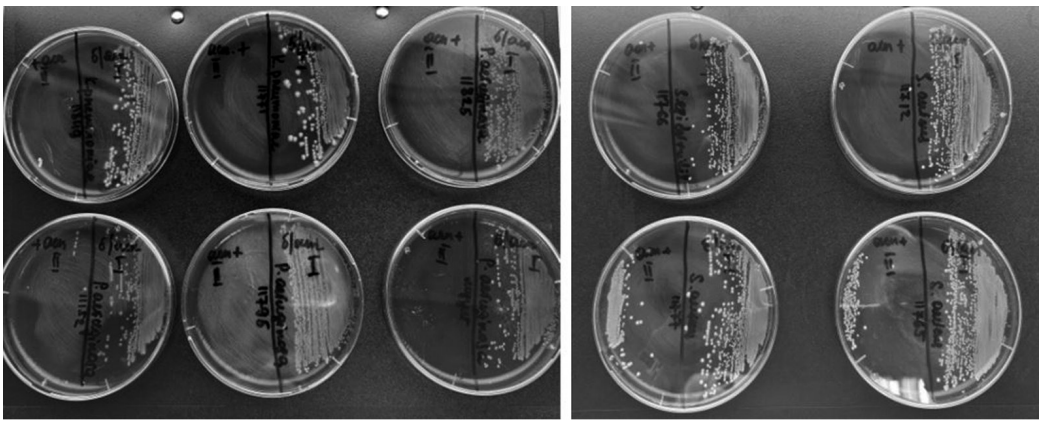
Antibacterial activity was assessed by the absence of a bacterial growth zone around the discs.

All experiments were performed in triplicate, and data are presented as mean (M) and standard deviation (SD). Statistical tests were performed using Gnumeric 1.12.17. Group comparisons were performed using Student's t-test after preliminary determination of sample distribution using the Anderson-Darling test. The significance threshold for all tests was $p < 0.05$.

This study was conducted as an in vitro study, without the participation of animals or humans.

RESULTS

Combined incubation of ASA (0.05 %) and gram-negative bacteria (K. *pneumoniae*, P. *aeruginosa*) did not show microbial growth on Petri dishes. In the case of S. *aureus* bacteria, the CFU count was reduced compared to the control group. There was no growth on the dishes containing S. *epidermidis* (Fig. 1).

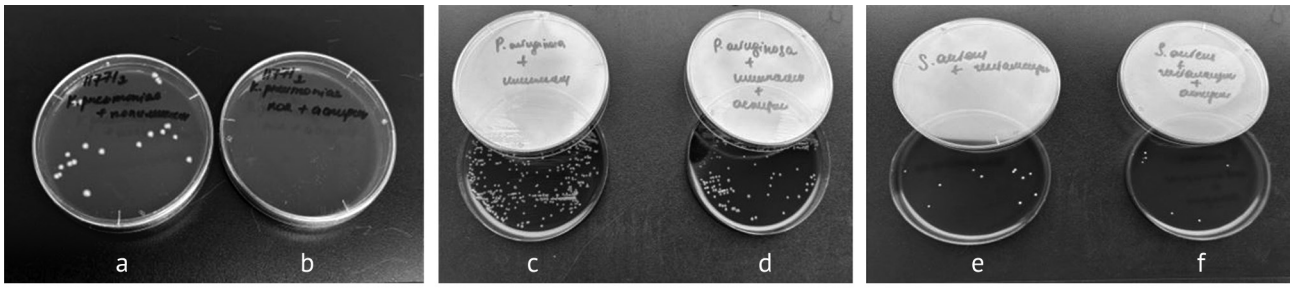


Gram-negative bacteria
(P. *aeruginosa*, K. *pneumoniae*)

Gram-positive bacteria
(S. *aureus*, S. *epidermidis*)

Fig. 1 Effect of ASA on growth of bacteria P. *aeruginosa*, K. *pneumoniae*, S. *aureus*, S. *epidermidis*. In dishes: control series is on the right and the series with ASA is on the left

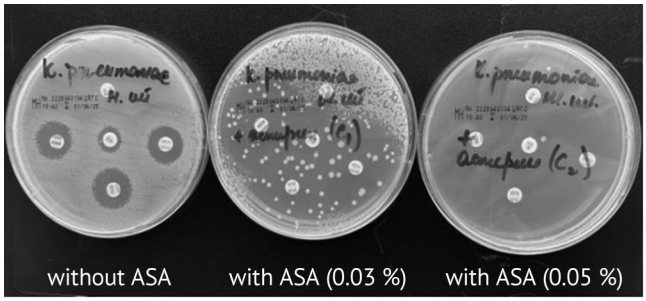
When polymyxin was co-incubated with P. *aeruginosa*, slight bacterial growth was observed in Petri dishes; only isolated colonies of were observed in the case of K. *pneumoniae*. Incubation of polymyxin and 0.03 % ASA showed no microbial growth of K. *pneumoniae* in dishes and slight growth was observed in the dishes with P. *aeruginosa*. Gentamicin inhibited the growth of S. aureus (a small number of bacteria were observed in the dishes). 0.03 % ASA enhanced the bactericidal properties of gentamicin (no bacterial growth was observed in the dishes) (Fig. 2).



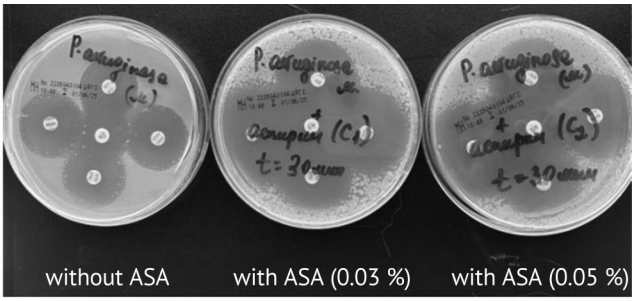
K. *pneumoniae* P. *aeruginosa* S. *aureus*

Fig. 2 Effect of ASA on the growth of microbial cultures K. *pneumoniae*, P. *aeruginosa*, S. aureus in blood agar after incubation with an antibiotic and a combination of an antibiotic with ASA: (a, c) polymyxin (5 mg/ml); (b, d) polymyxin (5 mg/ml) + ASA (0.03 %); (d) gentamicin (40 mg/ml); (e) gentamicin (40 mg/ml) + ASA (0.03 %)

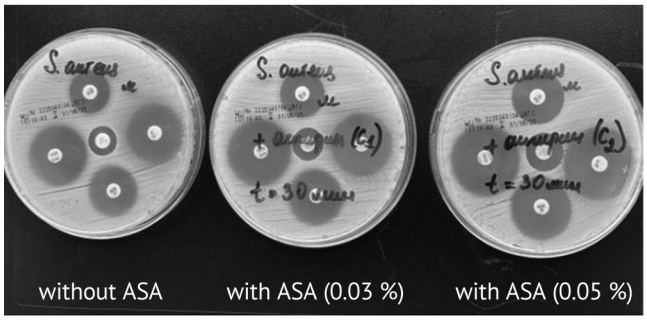
Differences in bacterial growth inhibition zones were also observed during antibiotic susceptibility tests that used different ASA concentration (Fig. 3 b, c). For K. *pneumomiae* strains and ASA (0.03 %) use, the bacterial growth inhibition zones did not differ from the control group; however, the number of viable cells on the agar surface was lower (Fig. 3 d).



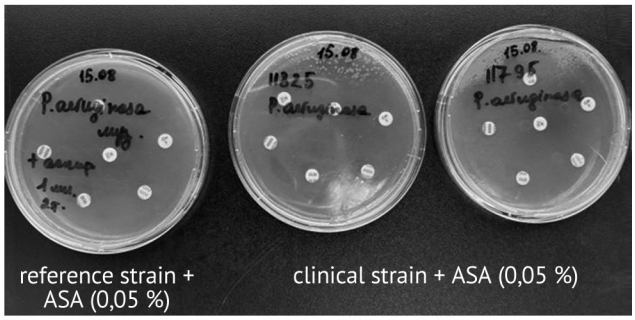
a — reference strain K. *pneumoniae*



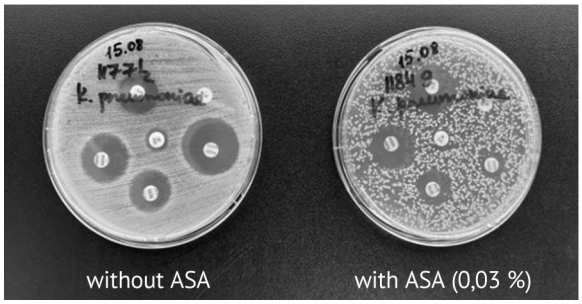
b — reference strain P. *aeruginosa*



c — reference strain S. *aureus*



d — examples of clinical strains P. *aeruginosa*



e — clinical strain K. *pneumoniae*

Fig. 3 Effect of ASA on the sensitivity of bacterial strains K. *pneumoniae*, P. *aeruginosa*, S. *aureus* to antibacterial drugs

In the ASA group, significant differences in growth inhibition zones were observed for clindamycin and cefoxitin compared with the control series for clinical S. *aureus* strains (Table 1). For S. *epidermidis* strains, differences in the growth inhibition zone were noted for clindamycin (Table 2). In dished with P. *aeruginosa* plates, significant changes in growth inhibition zones were observed for imipenem, meropenem, cefepime, and ciprofloxacin (Table 3).

Table 1

Diameter of the bacterial growth inhibition zone for S. *aureus*

Drug (mcg)	Diameter of the bacterial growth inhibition zone S. *aureus*, mm	
	Control series, *n* = 12	ASA (0.05 %), *n* = 12
Clindamycin (2)	25 ± 2	32 ± 2*
Cefoxitin (30)	29 ± 3	33 ± 2*
Vancomycin (5)	19 ± 1	19 ± 1
Gentamicin (30)	23 ± 2	24 ± 2
Ciprofloxacin (5)	25 ± 3	25 ± 2
Erythromycin (15)	26 ± 2	28 ± 3

Note: *n* — number of strains, * — significance of differences between the control series and the experimental series with ASA, $p < 0.05$

Table 2

Diameter of the bacterial growth inhibition zone for S. *epidermidis*

Drug (mcg)	Diameter of the bacterial growth inhibition zone S. *epidermidis*, mm	
	Control series, *n* = 12	ASA (0.05 %), *n* = 12
Clindamycin (2)	24 ± 2	29 ± 2*
Cefoxitin (30)	25 ± 2	26 ± 2
Vancomycin (5)	19 ± 1	19 ± 1
Gentamicin (30)	25 ± 3	24 ± 2
Ciprofloxacin (5)	26 ± 2	25 ± 2
Erythromycin (15)	25 ± 2	27 ± 2

Note: *n* — number of strains, * — significance of differences between the control series and the experimental series with ASA, $p < 0.05$

Table 3

Bacterial growth inhibition zone for P. *aeruginosa*

Antibacterial drug (mcg)	Bacterial growth inhibition zone P. *aeruginosa*, mm	
	Control series, *n* = 12	ASA (0.05 %), *n* = 12
Imipenem (10)	24 ± 3	30 ± 2*
Meropenem (10)	29 ± 2	33 ± 1*
Cefepime (30)	28 ± 3	32 ± 2*
Piperacillin/tazobactam (30/6)	26 ± 2	26 ± 3
Amikacin (30)	22 ± 2	25 ± 2
Ciprofloxacin (5)	28 ± 2	33 ± 2*

Note: *n* — number of strains, * — significance of differences between the control series and the experimental series with ASA, $p < 0.05$

DISCUSSION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as anti-inflammatory, analgesic, and antipyretic medications. However, there is evidence of the effects of NSAIDs on bacteria and associated infections [13]. Data on the in vitro activity of NSAIDs against planktonic bacteria and biofilms are currently contradictory [14–17].

One of the NSAIDs, acetylsalicylic acid, is a synthetic derivative of salicylic acid. ASA has a complex action that depends on its dosage. Its anti-inflammatory, antipyretic, and analgesic effects (medium/high doses of ~500–1000 mg are used) are mediated by inhibiting cyclooxygenase enzymes (COX-1 and COX-2), which are involved in the synthesis of prostaglandins which are substances that cause pain and swelling during inflammation. Its antiplatelet effect (low doses of ~75–100 mg are used) is mediated by blocking COX-1 in platelets.

Various studies have reported data on the effect of ASA on platelets after exposure to bacteria (Staphylococcus *aureus*, Enterococcus *faecalis*, Streptococcus *pyogenes*, Escherichia *coli*) [18, 19].

It has been shown that clinical strains of S. *aureus* can cause platelet activation, expression of cell death markers, and release of inflammatory factors [18, 19]. Acetylsalicylic acid limits the effect of S. *aureus* on platelets by reducing the rate of cell death, restoring their numbers, and decreasing the contribution of platelets to inflammation associated with the presence of bacterial pathogens in the blood.

Currently, the ability of aspirin to have an effect on the formation of biofilms by microorganisms, to exhibit synergism with antibiotics thus increasing the permeability of bacterial cell walls and making them more vulnerable, to modulate the body's immune response are being studied [8-12]. Kupferwasser et al concluded that the sensitivity of *MRSA* to ß-lactam antibiotics increases in the presence of aspirin [9]. Another study demonstrated an increased activity of vancomycin against resistant enterococci in the presence of aspirin [20]. A number of studies provide data on the bacteriostatic effect of ASA on gram-positive bacteria and the absence of any effect on gram-negative bacteria [13]. El-Mowafy et al observed a decrease in bacterial motility when studying the QS-inhibitory effect of ASA on P. *aeruginosa* [21]. Another study showed that exposure to ASA for 1.5 hours resulted in the removal of 30 % of biofilms formed by S. *epidermidis* and P. *aeruginosa* on polystyrene [22].

There are data in the literature that confirm the formation of resistant bacterial populations after exposure to NSAIDs, including ASA [14, 23].

In our study, the lack of growth in the dish with S. *epidermidis* bacteria in the presence of ASA, even without antibiotics, suggests that one mechanism of action may be the suppression of adhesion and biofilm formation [24]. ASA's pronounced effect on gram-negative bacteria (K. *pneumoniae*, P. *aeruginosa*) may be due to its impact on the outer membrane of microbial cells. However, the drug had a lesser effect on gram-positive bacteria (S. *aureus*) by only reducing metabolic activity. Aspirin, being a weak acid, can also disrupt membrane integrity and increase membrane permeability [8].

Our results suggest that ASA enhances the activity of polymyxin. The enhanced bactericidal activity of gentamicin in the presence of ASA against S. *aureus* may be related to the ability of salicylates to suppress protein synthesis and disrupt bacterial energy metabolism, which increases the efficiency of aminoglycoside accumulation within the cell [13].

This study is primarily descriptive in nature. Future plans include determining the MIC (minimum inhibitory concentration) and FIC (fractional inhibitory concentration) indices to confirm synergism, studying the effect of ASA on mature biofilms, and evaluating the effectiveness of the combination in relevant in vivo osteomyelitis models.

CONCLUSION

Our study demonstrated that acetylsalicylic acid exhibits synergism with a number of antibiotics in vitro and significantly enhances their bactericidal activity against clinical isolates associated with osteomyelitis.

Conflict of interests *Not declared.*

Funding source *Not declared.*

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