

Original article

<https://doi.org/10.18019/1028-4427-2022-28-6-788-793>**Analysis of the qualitative and quantitative community composition of bacteria isolated from the purulent focus in patients with chronic osteomyelitis over a three year period**Irina V. Shipitsyna^{1✉}, Elena V. Osipova²

Ilizarov National Medical Research Centre for Traumatology and Orthopedics, Kurgan, Russian Federation

¹ IVSchimik@mail.ru, <https://orcid.org/0000-0003-2012-3115>² E-V-Osipova@mail.ru, <https://orcid.org/0000-0003-2408-4352>**Abstract**

Introduction Annual microbiological monitoring of the leading causative agents of osteomyelitis and their antibiotic sensitivity is essential for identifying drugs that have lost the effectiveness. An increase in microbial associations requires different approaches to antibiotic therapy. Analysis of the composition of associations with a priority pathogen to be identified to avoid administration of ineffective drugs and optimize treatment. **The purpose** was to monitor qualitative and quantitative community composition of microorganisms isolated from the osteomyelitic focus in patients with chronic osteomyelitis over a three-year period. **Material and methods** The object of the study were strains of gram-negative and gram-positive bacteria isolated during primary inoculation as part of associations of bacteria from wounds and fistulas of patients who were treated in the clinic of infection osteology at the Kurgan Ilizarov Centre between 2018 and 2020. Standard bacteriological methods were used to isolate pure cultures. Bacteria were identified using bacteriological analyzer. **Results and discussion** Two-component microbial associations isolated in patients with chronic osteomyelitis included *P. aeruginosa* + *S. aureus*, *Enterobacteriaceae* + *S. aureus*, *S. aureus* + CoNS. The strains of *S. aureus* and *P. aeruginosa* were most common pathogens identified in mixed cultures. Inoculations of *S. aureus* + *Enterococcus* sp. increased and *P. aeruginosa* + *Enterococcus* sp. associations showed a two-fold decrease in 2020 compared to 2018. Three- and four-component associations of bacteria increased with the spectrum of combinations being diverse among the isolated mix cultures over a three-year period. Bacteria of the *Enterobacteriaceae* and *S. aureus* family were most common in three-component associations. Four-component associations were represented by mix cultures of gram-positive and gram-negative bacteria including NFGOB and *S. aureus*. **Conclusion** An increased frequency of isolated microbial associations necessitates an annual analysis of changes in the qualitative and quantitative composition to identify the spectrum of the most common microflora of the osteomyelitic focus and correct antibiotic therapy.

Keywords: chronic osteomyelitis, bacterial associations, biofilm, antibiotics, resistance

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INTRODUCTION

Staphylococci and gram-negative opportunistic bacteria are implicated in most patients with chronic osteomyelitis [1-6]. In recent years, there has been an increase in the proportion of pathogen associations, that are dominated by gram-positive cocci and gram-negative aerobic rods [5-8]. In, bacterial associations are common for patients with chronic osteomyelitis and long periods of fracture healing and defect replacement [8]. Intermicrobial interactions in associations have a significant impact on the forms and course of osteomyelitis [6, 7, 9]. There are data on the multidrug resistance of associated microbes that can be associated with the production of adaptive enzymes that destroy the antibacterial drug, and with the

ability of microorganisms to exist in the biofilm [10-17]. Annual microbiological monitoring of common pathogens of osteomyelitis and the antibiotic sensitivity is essential for identifying drugs that have lost the effectiveness. An increase in the number of microbial associations requires other approaches to antibiotic therapy. Composition of associations with a priority pathogen is to be identified to avoid administration of ineffective drugs and optimize treatment.

The purpose was to monitor qualitative and quantitative community composition of microorganisms isolated from the osteomyelitic focus in patients with chronic osteomyelitis over a three-year period.

MATERIAL AND METHODS

The study is aimed at exploring strains of gram-negative and gram-positive bacteria isolated during primary cultures as part of bacterial associations from wounds and fistulas of patients treated at the clinic of infection osteology, the Kurgan Ilizarov Centre, between

2018 and 2020. Standard methods were used to isolate pure cultures. Bacteria were identified on gram-negative NBC 44 and gram-positive PBC44 panels (WalkAway-40 Plus, Siemens). Digital data were processed using the AtteStat computer program, version 13.0.

RESULTS

In 2018, 821 strains were isolated as part of associations in bacteriological cultures from a purulent focus that accounted for 35.0 % of the total clinical isolates. There were 580 (22.9 %) and 559 (36.6 %) strains isolated in 2019 and 2020, respectively. Mix cultures were represented by two-, three- and four-component microbial associations. There were mostly two-component associations (78.0-82.8 %) with less three- and four-component associations measuring 15.8-19.2 % and 0.4-2.8 %, respectively (Fig. 1).

S. aureus strains predominated among clinical isolates in two-component associations (Table 1). Associations of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were most common in 2018 with the occurrence decreased by 1.6 times in 2020. The largest percentage accounted for associations *S. aureus* + *Enterococcus sp.* (18.5 %) in 2020 with *S. aureus* + coagulase-negative staphylococci (CoNS) decreased as compared to 2018. Mixed cultures of *S. aureus* + *Enterobacteriaceae* ranged between 8.4 and 12.9 % during the three-year period.

Associations formed by non-fermenting gram-negative bacteria (NFGNB) were second in frequency of occurrence (Table 2). A higher percentage of isolated mix cultures of NFGNB with *S. aureus* was noted in 2018 with 92 % accounting for associations of *P. aeruginosa* and *S. aureus*. Mixed cultures of *P. aeruginosa* in association with enterobacteria and enterococci were next in frequency of isolation.

Associations of *Enterobacteriaceae* family and *Staphylococcus sp.* or NFGOB were the third in frequency of isolated pathological material of the osteomyelitic focus (Table 3). The number of *Enterobacteriaceae* + *Enterobacteriaceae* mixed cultures increased by 5.1 % in 2020 as compared to 2018.

S. epidermidis strains were mostly isolated in association with *S. aureus* (Table 4). The number of *P. aeruginosa* + *S. epidermidis* decreased by 2.2 times over a three-year period. The isolated mixed cultures of epidermal staphylococcus with gram-positive or gram-negative bacteria showed slight changes.

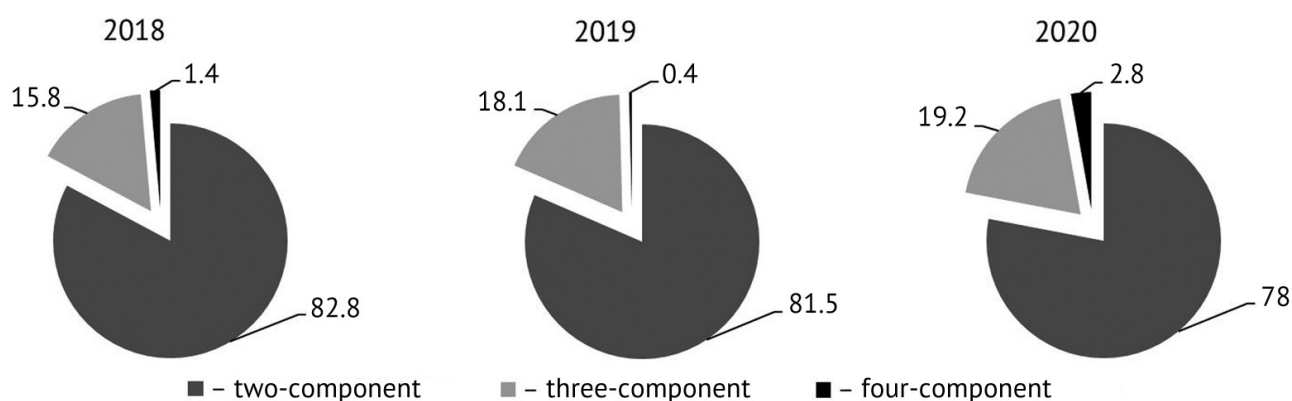


Fig. 1 Ratio of bacterial associations isolated between 2018 and 2020 (%)

Table 1

Qualitative and quantitative composition of two-component associations of *S. aureus* with gram-positive and gram-negative bacteria

| <i>S. aureus</i> + | Quantitative composition | | |
|--|--|-------------|-------------|
| | 2018 | 2019 | 2020 |
| | quantity (share of mix-cultures from TCA, %) | | |
| Total number of two-component associations (TCA) | 308 | 216 | 195 |
| CoNS | 34 (11 %) | 30 (13.9 %) | 16 (8.2 %) |
| <i>Enterococcus sp.</i> | 25 (8.1 %) | 21 (9.7 %) | 36 (18.5 %) |
| <i>Streptococcus sp.</i> | 12 (3.8 %) | 10 (4.6 %) | 18 (9.2 %) |
| <i>Corynebacterium sp.</i> | 1 (0.3 %) | – | – |
| <i>Acinetobacter sp.</i> | 4 (1.3 %) | 9 (4.2 %) | 3 (1.5 %) |
| <i>P. aeruginosa</i> | 46 (14.9 %) | 29 (13.4 %) | 18 (9.2 %) |
| <i>Enterobacteriaceae</i> | 26 (8.4 %) | 28 (12.9 %) | 21 (10.8 %) |

Table 2

Qualitative and quantitative composition of two-component associations of NFGNB with gram-positive and gram-negative bacteria

| NFGNB + | Quantitative composition | | |
|--|--|--------------------|--------------------|
| | 2018 | 2019 | 2020 |
| | quantity (share of mix-cultures from TCA, %) | | |
| Total number of two-component associations (TCA) | 308 | 216 | 195 |
| <i>S. aureus</i> | 50 (16.2 %) | 38 (17.6 %) | 21 (10.8 %) |
| <i>CoNS</i> | 22 (7.1 %) | 7 (3.2 %) | 8 (4.1 %) |
| <i>Enterococcus sp.</i> | 27 (8.8 %) | 7 (3.2 %) | 8 (4.1 %) |
| <i>Streptococcus sp.</i> | – | 2 (0.9 %) | – |
| <i>Enterobacteriaceae</i> | 30 (9.7 %) | 18 (8.3 %) | 18 (9.2 %) |
| <i>NFGNB</i> | 1 (0.3 %) | – | 1 (0.5 %) |
| <i>Corynebacterium sp.</i> | – | – | 1 (0.5 %) |

Table 3

Qualitative and quantitative composition of two-component associations of *Enterobacteriaceae* with gram-positive and gram-negative bacteria

| <i>Enterobacteriaceae</i> + | Quantitative composition | | |
|--|--|--------------------|--------------------|
| | 2018 | 2019 | 2020 |
| | quantity (share of mix-cultures from TCA, %) | | |
| Total number of two-component associations (TCA) | 308 | 216 | 195 |
| <i>Staphylococcus sp.</i> | 41 (13.3 %) | 35 (16.2 %) | 28 (14.4 %) |
| <i>Enterococcus sp.</i> | 20 (6.5 %) | 25 (11.6 %) | 16 (8.2 %) |
| <i>Streptococcus sp.</i> | 6 (1.9 %) | 1 (0.5 %) | – |
| <i>Corynebacterium sp.</i> | 2 (0.6 %) | 2 (0.9 %) | 1 (0.5 %) |
| <i>NFGNB</i> | 30 (9.7 %) | 18 (8.3 %) | 18 (9.2 %) |
| <i>Enterobacteriaceae</i> | 11 (3.6 %) | 6 (2.8 %) | 17 (8.7 %) |

Table 4

Qualitative and quantitative composition of two-component associations of *CoNS* with gram-positive and gram-negative bacteria

| <i>CoNS</i> + | Quantitative composition | | |
|--|--|-------------------|-------------------|
| | 2018 | 2019 | 2020 |
| | quantity (share of mix-cultures from TCA, %) | | |
| Total number of two-component associations (TCA) | 308 | 216 | 195 |
| <i>S. aureus.</i> | 34 (11.0 %) | 30 (9.7 %) | 16 (8.2 %) |
| <i>CoNS</i> | 9 (2.9 %) | 6 (2.8 %) | 3 (1.5 %) |
| <i>Enterococcus sp.</i> | 10 (3.2 %) | 4 (1.9 %) | 5 (2.7 %) |
| <i>Corynebacterium sp.</i> | 1 (0.2 %) | – | 1 (0.5 %) |
| <i>Streptococcus sp.</i> | 6 (1.8 %) | 4 (1.9 %) | 3 (1.5 %) |
| <i>Acinetobacter sp.</i> | 8 (2.6 %) | 3 (1.4 %) | 4 (2.1 %) |
| <i>P. aeruginosa</i> | 14 (4.5 %) | 4 (1.9 %) | 4 (2.1 %) |
| <i>Enterobacteriaceae</i> | 15 (4.9 %) | 7 (3.2 %) | 7 (3.7 %) |

Three-component associations were represented by bacteria of the *Staphylococcus sp.* family in combination with *Enterobacteriaceae* and NFGNB (Table 5). The number of *P. aeruginosa* + *Enterobacteriaceae* + *E. faecalis*; *Enterobacteriaceae* + *Enterobacteriaceae* + *Staphylococcus sp.* associations increased over the three-year period.

The total number of four-component associations was insignificant during the observation period. The associations included bacteria of the *Staphylococcus* family (Table 6). Other common microorganisms in such associations included NFGNB with a predominance of *P. aeruginosa* strains and bacteria of the *Enterobacteriaceae* family: *K. pneumoniae*, *E. coli*.

Table 5

Qualitative composition of three-component associations

| Associations | Quantity | | |
|--|-----------|-----------|-----------|
| | 2018 | 2019 | 2020 |
| <i>S. aureus</i> + CoNS + <i>P. aeruginosa</i> | 1 | 1 | – |
| <i>Staphylococcus</i> sp. + <i>P. aeruginosa</i> + <i>Enterococcus</i> sp., | 5 | 7 | 4 |
| CoNS + CoNS + <i>P. aeruginosa</i> | 1 | – | – |
| <i>Staphylococcus</i> sp. + <i>P. aeruginosa</i> + <i>Streptococcus</i> sp. | 1 | – | – |
| <i>P. aeruginosa</i> + <i>E. faecalis</i> + <i>Streptococcus</i> sp. | – | – | 1 |
| <i>Staphylococcus</i> sp. + <i>P. aeruginosa</i> + <i>Enterobacteriaceae</i> | 9 | 6 | 3 |
| <i>P. aeruginosa</i> + <i>Enterobacteriaceae</i> + <i>E. faecalis</i> | 3 | 6 | 11 |
| <i>P. aeruginosa</i> + <i>Enterobacteriaceae</i> + <i>Enterobacteriaceae</i> | 2 | 2 | – |
| <i>P. aeruginosa</i> + <i>Acinetobacter</i> sp. + <i>Enterobacteriaceae</i> | 2 | 1 | – |
| <i>Staphylococcus</i> sp. + <i>P. aeruginosa</i> + <i>A. baumannii</i> | 1 | 1 | 3 |
| <i>Staphylococcus</i> sp. + <i>Acinetobacter</i> sp. + <i>Enterococcus</i> sp. | 3 | – | 1 |
| <i>Staphylococcus</i> sp. + <i>Acinetobacter</i> sp. + <i>Streptococcus</i> sp. | – | 1 | – |
| <i>Staphylococcus</i> sp. + <i>Acinetobacter</i> sp. + <i>Enterobacteriaceae</i> | 3 | 4 | 1 |
| <i>Acinetobacter</i> sp. + <i>Enterobacteriaceae</i> + <i>Enterococcus</i> sp. | 5 | 2 | – |
| <i>Acinetobacter</i> sp. + <i>Enterobacteriaceae</i> + <i>Enterobacteriaceae</i> | 1 | 1 | – |
| <i>S. aureus</i> + CoNS + <i>Enterobacteriaceae</i> | 2 | 2 | 2 |
| <i>Enterobacteriaceae</i> + <i>Enterobacteriaceae</i> + <i>Enterobacteriaceae</i> | 1 | 1 | – |
| <i>Staphylococcus</i> sp. + <i>Streptococcus</i> sp. + <i>Enterobacteriaceae</i> | 2 | 1 | 3 |
| <i>Staphylococcus</i> sp. + <i>Enterococcus</i> sp. + <i>Enterobacteriaceae</i> | 3 | 3 | 5 |
| <i>Enterobacteriaceae</i> + <i>Enterobacteriaceae</i> + <i>Enterococcus</i> sp. | 3 | 4 | 3 |
| <i>Staphylococcus</i> sp. + <i>Enterobacteriaceae</i> + <i>Enterobacteriaceae</i> | 6 | 2 | 9 |
| CoNS + CoNS + <i>Enterobacteriaceae</i> | – | – | 1 |
| <i>Staphylococcus</i> sp. + <i>Enterobacteriaceae</i> + <i>Corynebacterium</i> sp. | 1 | – | – |
| <i>S. aureus</i> + CoNS + <i>Streptococcus</i> sp. | 1 | 2 | 1 |
| <i>S. aureus</i> + CoNS + CoNS | 1 | – | – |
| CoNS + CoNS + <i>Enterococcus</i> sp. | 2 | – | – |
| CoNS + CoNS + <i>Streptococcus</i> sp. | – | 1 | – |
| Total: | 59 | 48 | 48 |

Table 6

Qualitative composition of four-component associations

| Associations | Quantity | | |
|---|----------|----------|----------|
| | 2018 | 2019 | 2020 |
| <i>S. aureus</i> + <i>P. aeruginosa</i> + <i>K. pneumoniae</i> + <i>P. mirabilis</i> | – | – | 1 |
| <i>S. aureus</i> + <i>P. aeruginosa</i> + <i>K. pneumoniae</i> + <i>E. faecalis</i> | – | – | 1 |
| <i>S. salivarius</i> + <i>P. aeruginosa</i> + <i>B. cepacia</i> + <i>A. baumannii</i> | – | – | 1 |
| <i>S. aureus</i> + <i>P. aeruginosa</i> + <i>M. morganii</i> + <i>S. mitis</i> | 1 | – | – |
| <i>S. aureus</i> + <i>P. aeruginosa</i> + <i>A. baumannii</i> + <i>E. faecalis</i> , | 1 | 1 | – |
| <i>S. aureus</i> + <i>S. epidermidis</i> + <i>P. aeruginosa</i> + <i>P. mirabilis</i> | 1 | – | – |
| <i>S. saprophyticus</i> + <i>P. aeruginosa</i> + <i>K. pneumoniae</i> + <i>E. coli</i> | 1 | – | – |
| <i>S. epidermidis</i> + <i>K. pneumoniae</i> + <i>P. mirabilis</i> + <i>E. faecalis</i> | 1 | – | – |
| <i>S. aureus</i> + <i>P. mirabilis</i> + <i>Streptococcus</i> sp. | – | – | 1 |
| <i>S. aureus</i> + <i>S. epidermidis</i> + <i>P. mirabilis</i> + <i>E. coli</i> | – | – | 1 |
| <i>S. epidermidis</i> + <i>A. baumannii</i> + <i>E. coli</i> + <i>Streptococcus</i> sp. β -gem. | – | – | 1 |
| <i>S. aureus</i> + <i>E. coli</i> + <i>Enterobacter</i> sp. + <i>Citrobacter</i> sp. | 2 | – | – |
| Total: | 7 | 1 | 6 |

Retrospective analysis of common associations of microorganisms in patients with chronic osteomyelitis indicated two-component associations of bacteria as commonly inoculated of a purulent focus: *P. aeruginosa* + *S. aureus*; *Enterobacteriaceae* + *S. aureus*, *S. aureus* + CoNS. Strains of *S. aureus* and *P. aeruginosa* were common pathogens seen in mixed cultures. The number of isolated *S. aureus* + *Enterococcus* sp. increased in 2020 as compared to 2018, while the *P. aeruginosa* + *Enterococcus* sp. associations showed a two-fold decrease. The proportions of three- and four-component associations of bacteria increased among the isolated mix cultures with diverse spectrum of combined bacteria over a three-year period. Bacteria of the *Enterobacteriaceae* and *S. aureus* family were common among three-component associations. Four-component associations were represented by mix-cultures of gram-positive and gram-negative bacteria, NFGNB and *S. aureus*, in particular.

DISCUSSION

The purpose of our study was to characterize the qualitative and quantitative composition of the associations of microorganisms isolated from the

osteomyelitic focus and compare the findings with the data reported by Russian and foreign authors. Bacteria of the *Staphylococcus* family being isolated from the

purulent focus as monoculture and in association with gram-negative bacteria in chronic osteomyelitis are the main causative agents of the disease [1, 6, 8, 16, 18]. The associations account for 25-30 % [1, 6, 9, 10, 18]. Microbial relationships are known to be variable in associations depending on the nature with one bacteria being able to enhance the action of virulence determinants of another pathogen [10, 16, 18]. Many authors report a more severe clinical course of polymicrobial infections [7, 9-10]. Our results are comparable with literature data. *S. aureus* and *P. aeruginosa* strains and mixed cultures were common pathogens reported in associations in our series and our previous studies in different years ranging between 22.9 and 36.6 % [6, 18]. Two-component associations of bacteria were most common in the microbiological analysis of the purulent focus. We observed an increase in three- and four-component associations of bacteria with diverse spectrum of microbial combinations compared with the findings reported by Terekhova R.P. et al. (2016). Our previous series showed different bacteria relationships in the associations including antagonistic, synergistic, or neutral associations that could affect the severity of the pathogenic properties of microorganisms [18].

The virulence of bacteria of the *Enterobacter* family is reported to be significantly increased in association with bacteria of the *Citrobacter* family [16]. Strains of *Proteus sp.* and *P. aeruginosa* as part of microbial associations have a pronounced proteolytic activity [16, 18]. The *Enterococcus sp.* bacteria can initiate an inflammatory

response in synergistic interactions with other bacteria without affecting the further course of the process [20]. The growth rate of microorganisms, pathogenicity and susceptibility to antibiotics can change due to interspecies relationships [19-23]. Our previous studies have shown that the composition of associations can change the sensitivity of microorganisms to antibacterial drugs [24]. There are more reports on reduced activity against staphylococci of beta-lactam antibiotics, macrolides, aminoglycosides and lincosamides [13, 24, 25]. Gram-negative microflora can be resistant to at least 8-10 different antimicrobial drugs [24, 26]. For example, strains of *Pseudomonas aeruginosa* can be simultaneously insensitive to cephalosporins, imipenem and meropenem, piperacillin/tazobactam, fluoroquinolones, and aminoglycosides [26-28]. The number of microbes resistant to antibacterial drugs – associates grows every year [28-31]. Biofilm, a three-dimensional biological structure that resists external and internal protection factors is reported to be one of the causes of bacterial resistance [13, 14, 29]. Populations of bacteria with different protective properties can be present inside the biofilm. Some strains within a biofilm can produce β -lactamases that can lead to the protection of other bacteria [29-30]. Ampicillin poorly penetrates into the biofilm formed by *K. pneumoniae* strains, while ampicillin, co-trimoxazole, and vancomycin penetrate into *Enterococcus faecalis* communities [30-34]. The effectiveness of standard antibiotic therapy in the treatment of such biofilm infections will be questionable.

CONCLUSION

An increased frequency of isolated microbial associations has shown the need for an annual analysis of changes in the qualitative and quantitative

composition to identify the spectrum of common bacteria of the osteomyelitic focus and adjust the antibiotic therapy.

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Information about the authors:

1. Irina V. Shipitsyna – Candidate of Biological Sciences, IVSchimik@mail.ru, <https://orcid.org/0000-0003-2012-3115>;
2. Elena V. Osipova – Candidate of Biological Sciences, E-V-Osipova@mail.ru, <https://orcid.org/0000-0003-2408-4352>.