MICROBIOLOGICAL SPECTRUM OF CAUSATIVE AGENTS OF IMPLANT-ASSOCIATED INFECTION IN THE TREATMENT OF COMPLICATIONS OF TRANSPEDICULAR FIXATION OF THE SPINE USING THE NEGATIVE PRESSURE METHOD

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Objective. To analyze the leading pathogens of implant-associated infection (IAI) after spinal surgery and identification of trends in the change in the pattern of microorganisms at the stages of treatment using the negative pressure method (NPWT systems).
Material and Methods. The results of microbiological cultures of 25 patients with IAI of the spine were studied. The frequency of occurrence of Gram-positive and Gram-negative pathogens was estimated. The leading pathogens in the species spectrum were identified. Changes in the structure of identified microorganisms were studied at various time intervals of treatment.
Results. A total of 136 microbiological studies were performed in 25 patients at the stages of treatment with the negative pressure method, with the identification of microorganisms in 127 (93.3 %) cases. The frequency of gram-negative microflora was 50.0 %, gram-positive – 42.6 %, *Candida sp.* – 0.7%, in 15.4 % microbial associations with the dominance of gram-negative microflora were identified at all periods of treatment. Not only the diversity of the isolated flora was noted (*E. faecalis* – 16.5 %, *P. aeruginosa* – 14.2 %, *K. pneumoniae* – 11.0 %, *S. aureus* and *A. baumannii* – 9.4 % each and *S. epidermidis* – 8.6 %), but also changes in the spectrum of flora at the stages of treatment: on the 1st and 2nd weeks from the first debridement intervention and the installation of the NPWT system, *E. faecalis* was most often detected, on the 3rd and 4th weeks – *P. aeruginosa* and *A. baumannii*, during the 2nd month – *E. faecalis* and *P. aeruginosa*, later – gram-negative bacteria against the background of an increase in the incidence of *K. pneumoniae*. The frequency of verification of other species of microorganisms had no statistically significant differences. Microbial associations were found from the 2nd month of treatment. The change in pathogens was noted in 72 % of cases during the treatment of IAI of the spine. On average, this treatment required 7–8 NPWT dressing changes per patient. This method of treatment made it possible to achieve both negative results of microbiological examination and stable relief of the infectious process.
Conclusion. IAI, which complicates surgical interventions on the spine, is characterized by a change in pathogens during treatment, which requires not only multiple debridement with the replacement of the NPWT dressing, but also adequate long-term rational (etiologically justified) antibacterial therapy, based on the control of data on both the spectrum and on microbial resistance.
Key Words: implant-associated infection, spine surgery, leading microorganisms, microbiological monitoring, postoperative complications in spine surgery, NPWT, negative pressure wound treatment.
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Increasing in number of spinal surgeries unavoidably results in an increase in infectious inflammatory complications, which include implant-associated infection (IAI).

According to various sources [1–4], the occurrence of IAI in spinal surgery varies from 0.7 to 20.0 % and today remains a major, not only clinical, but also organizational challenge. The possibility of full-fledged treatment of IAI is available not in all hospitals where surgical treatment of spinal pathology is performed. Moreover, the progression of infection considerably lengthens the hospital stay, deteriorates the financial ratio of the medical institution [5] and negatively affects the quality of life of the patient [6–8].

The vast majority of cases of infectious complications in spinal surgery are diagnosed in the first 10 days from the moment of elective surgery. The early infections are assumed to occur in the period from 3 days to 3 months after surgery [1, 4], and the late ones occur in more distant terms.

Treatment of IAI is associated with a number of challenges, particularly when the possible removal of surgical hardware poses a threat to the development of neurological and secondary orthopedic complications. Negative pressure wound treatment (the NPWT system and its modifications) is among the techniques that promises to hope for the relief of IAI while maintaining the inserted implants. Nowadays, indications for the use of this technique are expanding [8]. Controlled negative pressure in the cavity of the wound ensures constant aspiration of the wound exudate, reduces the size of the wound, improves regional blood flow [8], and, as a result, promotes the formation of granulation tissue and reduces microbiological contamination [9].

As with any orthopedic infection, the treatment of spinal IAI requires, in addition to debridement, the use of appropriate antibiotic therapy based on the identification of microflora and its drug susceptibility. Moreover, if the frequency of detecting different flora, according to publications [10–14], varies in a large range, then the predominant pathogens of such infections are *Staphylococcus aureus/MRSA* (frequency 18.9–33.1 % in the structure of pathogens), *Staphylococcus epidermidis/MRSE* (13.3–29.5 %), and *Pseudomonas aeruginosa* (8.7–12.7 %).

The objective is to analyze the leading pathogens of implant-associated infection (IAI) of spine and identify trends in the change in the pattern of identified microorganisms at the various stages of treatment using the negative pressure method (NPWT systems).

Material and Methods

The analysis includes data from microbiological studies of patients selected by continuous screening from the overall array of patients treated in the unit for the consequences of musculoskeletal injuries and osteoarticular infection between 2016 and 2021.

Inclusion criteria:

1) surgical intervention on the spine (thoracic and lumbar spine), performed with the use of transpedicular fixation;

2) the use of the negative pressure method (NPWT system) to treat developed infectious surgical wound complications;

3) follow-up for at least 8 months after relief of the infectious process.

Exclusion criteria consisted of:

1) the use of inflow-outflow drainage in the treatment of infectious complication;

2) the presence of non-infectious surgical wound complications;

3) the presence of intraoperative complications during the original (aseptic) surgery.

Patients

The study design corresponds to a selective case series and includes the findings of microbiological studies conducted on 25 patients (17 (68 %) women and 8 (32 %) men) who were admitted to the unit within 12 days of the IAI development. The structure of the underlying disease, for which the elective surgical intervention was performed, is represented by spinal deformities in 4 (16 %) cases, by degenerative and dystrophic diseases in 9 (36 %), by fractures of vertebral bodies in 8 (32 %), and by spondylitis in 4 (16 %).

Patients suffering from spondylitis and its primary (prior to the start of NPWT therapy) treatment were characterized by the following features:

– in all cases, spondylitis was endogenous (community-acquired) and chronic in the acute phase;

– morphologically and anatomically, spondylitis was localized in the thoracic vertebral bodies (at no more than one level);

– the primary intervention consisted of two stages: the first one – transpedicular fixation through the posterior approach, then (the second stage) – vertebral body resection with its substitution by a mesh interbody implant with autogenous bone;

– the intervention was performed in the acute phase; when a patient complains of pain in the corresponding region of the spine and an elevation of inflammatory markers (C-reactive protein, erythrocyte sedimentation rate (ESR), and fibrinogen) according to laboratory tests; in the absence of the effect of the use of broad-spectrum antibiotics;

– the infectious process was confirmed bacteriologically by culture-based examination of tissue samples obtained during resection of the affected vertebral bodies.

The average age of patients was 52 (min – 12; max – 74). Early infection of the surgical site was diagnosed in 23 (92 %) patients, late infection – in 2 (8 %) patients with an average duration of complications of 10.36 days after the primary intervention (min – 5, max – 18).

Techniques

During the primary intervention, all patients had bilateral (paravertebral left and right) drains inset into the surgical wound.

During the progression of IAI, clinical, X-ray (if necessary, CT, MRI and ultrasound investigations), laboratory and microbiological studies were performed. Since there were surgical wounds in all cases, the collection of wound exudate for culturing was performed during the first revision surgery, as well as during each subsequent one. Sampling was done from several different sites (from 3 to 5); in case of surgical hardware removal, the material from the bed of the screws and the screws themselves were also collected for microbiological testing. The removed screws were treated with ultrasound in a special bath using the Söring Sonoca device (Germany) to further destroy biofilms and improve the quality of microbiological diagnostics. Surgical treatment of each patient included the following stages: excision of devitalized and modified soft and bone tissues; debridement of a surgical wound using a pulsating jet (pulse lavage) washing system with a large volume of antiseptic solution (from 4 liters); the NPWT system was inserted followed by debridement interventions every 3–5 days with the replacement of polyurethane foam sponges, adhesive films, and a port until the moment of stable relief of the infectious and inflammatory process and the beginning of sustainable healing of the postoperative wound. Removal of the surgical hardware was performed only when it was unstable, which was performed in 2 (8.0 %) of 25 follow-up on the 20th and 48th days after the start of NPWT.

Seven to eight elective surgeries were performed on average per patient to replace the components of the NPWT dressings. The decision to remove the NPWT system was made individually for each patient under the achievement of the following targets:

– negative results of bacteriological studies on the background of normalization of complete and biochemical blood assays, including inflammatory parameters (ESR, C-reactive protein, and fibrinogen);

– absence of inflammatory lymph in wound exudate;

– visual (during the change of the NPWT dressing) signs of complete cleansing and healing of the surgical wound;

– reducing the wound size (including by means of its layered partial closure) to a size that allowed for final closure with minimal risk: no more than subcutaneous tissue in depth; no more than 7 cm in length.

Pathogen cultivation was performed according to the standard procedure [15]. The sampling for the study was performed using intraoperative smears; genus-specific and taxonomic relationships of microorganisms and their sensitivity to antibiotics were determined using the Vitec 2 compact microbial detection system (BioMerieux, France).

The study considered all the findings of intraoperative microbiological cultures performed on average with an interval of 4–5 days until the time of stable relief of the inflammatory process, except for the final (before discharge) negative control studies, accompanied by the above-listed signs of infection process relief. If two or more microorganisms were detected in one culture, the pathogens were registered separately. The outcomes were recorded in the following control periods: 1, 2, 3 and 4 weeks; 2 months (results 5–8 weeks); 3 months (9–12 weeks); and 4 months (13–16 weeks) from the start of treatment. The number of cases of detection for both gram-positive and gram-negative microorganisms was recorded at various stages of the study.

In the postoperative period, all patients underwent infusion, anti-inflammatory and antibacterial therapy, which was prescribed considering the results of pathogen sensitivity tests. Combined empiric treatment with drugs from the classes of glycopeptides and fluoroquinolones was used in case of culture-negative infection (9 tests).

Statistical analysis

Statistical data processing was performed using the IBM SPSS Statistics 26 software with the following methods:

– descriptive statistics (minimum, maximum, mean, standard deviation) – for quantitative data; the proportion of polymicrobial infections at various control periods was described separately;

– the incidence of certain microorganisms, as well as the structure of their spectrum (in percent);

– Fisher's two-tailed exact test was used for statistical comparison of the incidence rates of the most clinically significant microorganisms, as well as gram-positive and gram-negative flora. The most significant microorganisms included: *Enterococcus faecalis, Klebsiella pneumoniae, methicillin-resistant strains of Staphylococcus aureus (MRSA)* and *Staphylococcus epidermidis (MRSE)*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, methicillin-sensitive isolates of *Staphylococcus aureus (MSSA)* and *Staphylococcus epidermidis (MSSE)*. P = 0.05 was chosen as the threshold value of statistical significance; the choice of the test is due to the small number (5 or less) of samples with a particular microorganism detected at each individual control period.

The study was performed in accordance with the ethical standards of the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects)" (with amendments as of 2013).

Results

A total of 136 microbiological studies were performed on 25 patients. Meanwhile, identification of isolates of microorganisms was done in 127 (93.4 %) cases. Gram-negative flora was found in 68 (50.0 %) isolates, gram-positive flora – in 58 (42.6 %) isolates and fungi – in 1 (0.7 %) isolate (Table 1).

During the entire treatment period, the same microorganism was isolated in 4 (16 %) patients. The change of microbial isolates was recorded in 18 (72 %) patients, moreover 13 (52 %) of them had up to 3 changes of microorganisms, and in 5 (20 %) of them had from 5 to 8 changes. Despite the obvious clinical picture of the infectious process, the pathogen was not found in 3 (12 %) patients, including repeated studies.

The species composition of pathogens revealed at each stage of treatment is given in Table 2.

E. faecalis prevailed among gram-positive bacteria: 21 (16.5 %) out of 127 isolates. The proportion of *S. aureus* in the structure of pathogens was 9.4 % (12 isolates), including *MRSA* (6.3 %) and *MSSA* (3.1 %). If the detection rate of *S. epidermidis* was 8.6 % (11 microorganisms), *MRSE* was identified in 3.1 %, and *MSSE* in 5.5 %. *Staphylococcus hominis* was found in 8 (6.3 %) cases, and *Staphylococcus haemolyticus* was found in 2 (1.6 %) cases. Rare cases of identification of *Corynebacterium*, *Staphylococcus capitis*, *Staphylococcus lentus* and *Staphylococcus warneri* have also been found.

The leading position among gram-negative bacteria was taken by *P. aeruginosa* – 18 (14.2 %) microisolates, followed by *K. pneumoniae* – 14 (11.0 %), *A. baumannii* – 12 (9.4 %); *Escherichia coli* and *Proteus sp.* were identified in 7 (5.5 %) and 8 (6.3 %) cases, respectively. *Enterobacter cloacae* and *Serratia marcescens* were detected with equal frequency – 3 (2.3 %) isolates each; verification of *Aeromonas hydrophila/caviae*, *Burkholderia cepacia* and *Pantoea dispersa* was single.

In general, the incidence of microbial associations was 15.4 % (21 samples at n = 136). This amount includes 2 (1.5 %) cases of gram-positive bacteria, 9 (6.6 %) cases of gram-negative bacteria and 10 (7.3 %) cases of coexistence of both types of microisolates. It is worth noting that initially only 2 (11.0 %) patients had a polymicrobial etiology of IAI, and later, during treatment, mixed infection was detected in 6 (33.3 %) patients. The distribution of polymicrobial infection in different periods of follow-up, together with the quantitative characteristics of microorganisms, is demonstrated in Fig. 1 and 2.

As a result, there is a shift in the flora associated with NPWT: *E. faecalis* dominates on the first and second weeks

(17.9 and 20.8 %, respectively), then it is replaced on the third week by *P. aeruginosa* (22.7 %) and on the fourth week by *A. baumannii* (40.0 %). With longer terms, the flora becomes more diverse: after 2 months, the proportion of *E. faecalis* and *P. aeruginosa* accounted for 18.5 % of microorganisms; during the 3rd month, the proportion of *K. pneumoniae*, *P. vulgaris*, *S. marcescens* and *MSSA* was 16.7 % for each; and during the 4th month, 30.8 % of positive cultures were accounted for by *A. baumannii* and *K. pneumoniae*.

The initial data for the incidence rates of microorganisms of the greatest clinical significance are given in Table 3.

Statistical analysis using Fisher's two-tailed exact test revealed the following: on the whole, no trends of significant changes are registered in the spectrum of microorganisms (Table 4), except for an increase in the incidence of *K. pneumoniae* ($p < 0.05$, typed in bold) by the 4th month.

Discussion

In spinal surgery, IAI, as a rule, manifests itself by the preservation of pain syndrome within 5–7 days after the primary elective surgery. It is hardly stopped by both NSAIDs and narcotic analgesics. Later, inflammatory changes develop in the surgical site, wound dehiscence or difficulties in healing its edges. In some cases, the onset of wound exudate of a different nature occurs: from seropurulent to hemorrhagic and purulent. As a rule, IAI is diagnosed at this stage. Nevertheless, defects in treatment policy (temporary delayed transfer to a specialized unit, inadequate antibacterial therapy, debridement surgeries at a "clean" vertebrology unit, insufficient compliance with the principles of septic surgery) delay the timely provision of specialized care. For this reason, patients were admitted to a specialized unit within up to 12 days from the moment of IAI development.

The analysis of microbiological monitoring of the examined patients showed the prevalence of gram-negative microorganisms in comparison with gram-positive ones: 68 (50.0 %) versus 58 (42.6 %) at n = 136, which distinguishes the microbiological profile of IAI after the replacement of large joints [16]. The obtained results differ from the data of other papers reporting the predominance of gram-positive microflora in the studied category of patients [13, 14, 17–20]. Its level reaches 60.4 % [19] with the predominance of *MRSA* and *MRSE* [19–21] with fluctuations in the incidence of gram-negative flora in the range from 25.7 to 30.5 % [13, 18, 19, 22].

In our study, the leading pathogens of IAI were *E. faecalis*, *P. aeruginosa* and *K. pneumoniae*, the proportion of which was 16.5, 14.2 and 11.0 %, respectively; isolates of *S. aureus* and *A. baumannii* were verified with equal incidence (9.4 % each, respectively) with a comparable specific gravity of *S. epidermidis* (8.6 %). Meanwhile, the ratio of methicillin-resistant and methicillin-sensitive strains of *S. aureus* was 2 : 1, *S. epidermidis* – about 1 : 2. Despite the increased incidence of methicillin-resistant strains of these species shown in other studies [12], no significant differences in the frequency of their detection were found. *S. hominis (MR)* and *Proteus sp.* were identified in 6.3 % of cases, *E. coli* in 5.5 %, and *E. cloacae* in 2.3 %.

The obtained results showed an inconsistency of the data with the papers of other authors, reporting mainly the predominance of representatives of *S. epidermidis* and *S. aureus* in IAI of the spine. For example, O.A. Smekalenkov et al. [12] present the indicators for *S. epidermidis* – 29.5 %, *S. aureus* – 21.1 %, while the incidence of *P. aeruginosa* was equal to 12.7 %, and *K. pneumoniae* – 7.1 %. In another article, the same authors [13] point to the preservation of epidermal staphylococcus as a leading position in the etiology of deep IAI, complicating spinal surgery, mainly due to the prevalence of *MRSE* (22.8 %). Meanwhile, the proportion of *MRSA* was 18.9 %, and *K. pneumoniae* increased to 13.9 %. D.N. Dolotin and M.V. Mikhailovsky [23] also marked the incidence of *S. epidermidis* in 68.0 % of cases, which is by 8 times higher than our indicator. A different picture is shown by O.G. Prudnikova et al. [14]: according to their data, obligate and anaerobic gram-positive *Cutibacterium acnes* (formerly *Propionibacterium acnes*) dominated in 42.8 %, while the proportion of *S. epidermidis* was 25.7 %.

Despite the different sampling and research design, in general, it can be considered that gram-positive flora, namely *Staphylococcus*, maintains a predominant position in the spectrum of identified microorganisms in the considered abnormality.

Data collected on the change in the profile of microbial agents detected during NPWT at different periods is of particular concern. The study showed statistically significant differences in the incidence of *K. pneumoniae* by the 4th month in comparison with the 1st and 2nd weeks of treatment. Notable is the absence of *MRSE* and *MRSA* in isolates after three weeks and two months of follow-up, respectively. This may indirectly testify to the efficacy of complex antibiotic therapy and debridement surgeries with the use of the NPWT system against these microorganisms.

The most late periods of incidence for *MSSA* and *MSSE* are the 3rd and 2nd months of follow-up, respectively, for *E. faecalis* – the 2nd month.

As can be seen from the data given above, during the entire follow-up period there was a shift from gram-positive to gram-negative flora. During the 1st and 2nd weeks, *E. faecalis* dominated; on the 3rd and 4th weeks – *P. aeruginosa* and *A. baumannii*; during the 2nd month, the frequency of identification of *E. faecalis* and *P. aeruginosa* bacteria was the same, then the representatives of gram-negative flora were identified.

Negative results of microbiological studies in the first week with the presence of clinical signs of an infectious process may indicate the presence of formed bacterial biofilms in the surgical wound. The latter are characterized by the phenomenon of culture negativity since such bacteria are not contained in planktonic forms and, consequently, do not form colonies on agar [24]. Later, culture-negative results are not found, which may be explained by the systematic destruc-

Table 1

Data on microorganisms detected at different time intervals, n (%)

Pathogen	The 1st week	The 2nd week	The 3rd week	The 4th week	The 2nd month	The 3rd month	The 4th month
A. baumannii	2 (7.1)	1 (4.2)	–	4 (40.0)	1 (3.7)	–	4 (30.8)
A. hydrophila/caviae	–	–	–	–	1 (3.7)	–	–
B. cepacia	–	1 (4.2)	–	–	–	–	–
Candida sp.	–	–	–	–	1 (3.7)	–	–
Corynebacterium sp	–	–	1 (4.5)	–	–	–	–
E. cloacae complex	–	1 (4.2)	1 (4.5)	–	–	–	–
E. cloacae ssp dissoivens	1 (3.6)	–	–	–	–	–	–
E. faecalis	5 (17.9)	5 (20.8)	4 (18.2)	2 (20.0)	5 (18.5)	–	–
E. coli	1 (3.6)	1 (4.2)	1 (4.5)	–	1 (3.7)	1 (8.3)	2 (15.4)
K. pneumoniae	1 (3.6)	1 (4.2)	1 (4.5)	2 (20.0)	3 (11.1)	2 (16.7)	4 (30.8)
MRSA	3 (10.7)	3 (12.5)	1 (4.5)	–	1 (3.7)	–	–
MRSE	1 (3.6)	1 (4.2)	2 (9.1)	–	–	–	–
P. dispersa	–	–	1 (4.5)	–	–	–	–
Proteus mirabilis	–	1 (4.2)	1 (4.5)	–	2 (7.4)	–	–
Proteus penneri	–	–	–	1 (10.0)	1 (3.7)	–	–
Proteus vulgaris	–	–	–	–	–	2 (16.7)	–
P. aeruginosa	2 (7.1)	2 (8.3)	5 (22.7)	1 (10.0)	5 (18.5)	1 (8.3)	3 (23.1)
S. marcescens	–	–	–	–	1 (3.7)	2 (16.7)	–
MSSA	1 (3.6)	1 (4.2)	–	–	–	2 (16.7)	–
S. capitis	–	1 (4.2)	–	–	–	–	–
MSSE	1 (3.6)	1 (4.2)	3 (13.6)	–	2 (7.4)	–	–
S. haemolyticus	2 (7.1)	–	–	–	–	–	–
S. hominis	1 (3.6)	2 (8.3)	1 (4.5)	–	3 (11.1)	1 (8.3)	–
S. lentus	–	–	–	–	–	1 (8.3)	–
S. warneri	1 (3.6)	–	–	–	–	–	–
None	6 (21.4)	2 (8.3)	1 (4.5)	–	–	–	–
Total	28 (100.0)	24 (100.0)	22 (100.0)	10 (100.0)	27 (100.0)	12 (100.0)	13 (100.0)

Table 2

Distribution of gram-negative and gram-positive flora

Flora	The 1st week	The 2nd week	The 3rd week	The 4th week	The 2nd month	The 3rd month	The 4th month
Gram-positive	10	9	7	0	6	4	0
Gram-negative	12	13	14	10	20	8	13
Other*	6	2	1	0	1	0	0
Total	28	24	22	10	27	12	13

* the 1st — 3rd week: total of 9 culture-negative studies; the 2nd month — 1 sample with detection of C*andida sp*.

tion of biofilms by pulse lavage systems during staged debridement interventions with the replacement of NPWT dressings. Even after the treatment of the removed implants by ultrasonic cavitation, culture-negative results were found in a number of cases.

We have identified microbial associations in 15.4 % of cases, which does not exceed the indicators of other papers [8]. Mixed infections were found almost equally. These infections involved both gram-positive and gram-negative microorganisms and gram-negative flora only, the proportion of which in our study was 7.3 and 6.6 %, respectively. The detection of associations represented by gram-positive bacteria only turned out to be rare for the IAI of the spine. They were found only in two cases during the entire follow-up period. *E. faecalis*, *P. aeruginosa*, *A. baumannii*, and *K. Pneumoniae* were the most committed to coexistence with other microbial agents, while *S. aureus* and *S. epidermidis* isolates were predominantly monomicrobial.

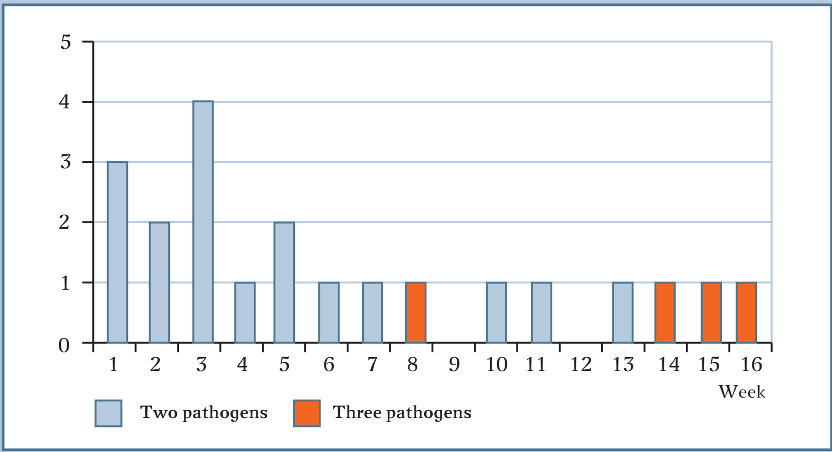

Fig. 1
Distribution of cases of polymicrobial infection by weeks

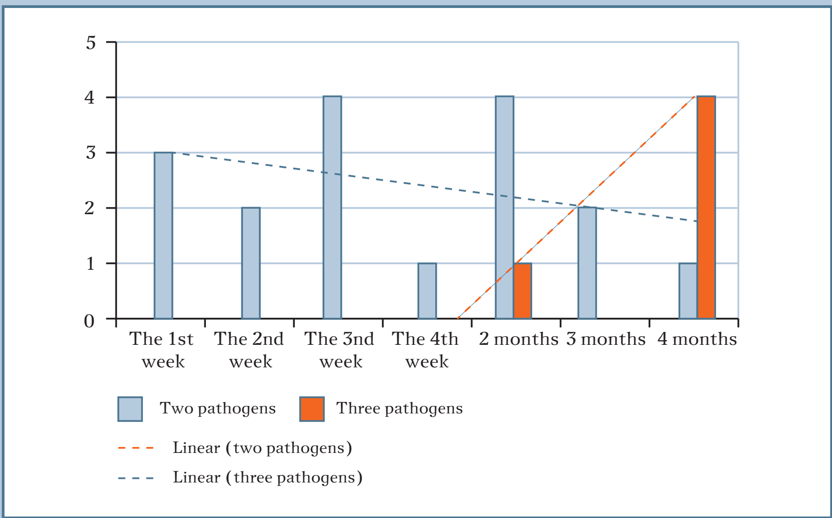

Fig. 2
Distribution of cases of polymicrobial infection according to the time intervals accepted in this study; trend lines are marked

Polymicrobial infection cases were observed at all periods of therapy. Nevertheless, during the first month (1–4 weeks), associations with only two pathogens were identified, while verification of mixed infections with three microorganisms increased from the 2nd month, with a marked increase in the number of such cases by the 4th month. Probably, this indicates the formation of a peculiar ecosystem of microorganisms in the wound being in relative equilibrium with each other.

According to the obtained results, the change of microorganisms in the course of treatment is observed in patients with IAI localized in the spine associated with multiple debridement interventions with the use of NPWT systems. For example, patients with three bacterial changes in the vast majority underwent 5 revision surgeries, and those with five or more changes – from 7 to 11 revision surgeries. If the change of microorganisms was not detected or was recorded once, polyresistant gram-negative isolates or mixed infections, including gram-positive and gram-negative microflora, were mainly identified. In these cases, it took from 4 to 7 surgeries for the complete relief of the infectious process and wound healing. Only one patient who had only *MRSE* identified throughout the treatment underwent 10 procedures due to the weak regenerative capacity of tissues.

The incidence of culture-negative infection, which was 12 %, is comparable to the data of other microbiological reports [22], in which it is estimated in the range from 11.1 to 34.5 %, and according to Lee et al. [21], reaches 40.6 %. The negative microbiological culture result with obvious clinical signs of infection can be explained not only by technical reasons (failure to comply with the rules for sampling the material and transporting it to the laboratory, non-compliance with cultivation techniques), but also by the existence of microbes in the composition of biofilms (biofilm infections) that are rarely found by traditional culture methods, the sensitivity of which is about 20–52 % [24–26]. Moreover, antibiotic therapy prior to the debridement surgery contributes to the eradication of planktonic forms of bacteria and can also lead to negative culture [17].

The microbiological picture in each individual medical institution or unit that treats infectious and inflammatory complications of spinal surgery may be characterized by distinctness depending on the flora and the established ecosystem. It is essential to perform continuous microbiological monitoring of spinal IAI pathogens to define appropriate treatment options and predict their outcomes [23].

It is advisable both to introduce molecular techniques of microbiological diagnostics and to expand the number of examined patients to obtain more accurate data on the microbiological picture of infectious complications in spinal surgery.

Table 3

Occurrence of individual microorganisms, n (%)

Pathogen	The 1st week	The 2nd week	The 3rd week	The 4th week	The 2nd month	The 3rd month	The 4th month
E. faecalis	5 (17.9)	5 (20.8)	4 (18.2)	2 (20.0)	5 (18.5)	—	—
MRSA	3 (10.7)	3 (12.5)	1 (4.5)	—	1 (3.7)	—	—
MRSE	1 (3.6)	1 (4.2)	2 (9.1)	—	—	—	—
MSSA	1 (3.6)	1 (4.2)	—	—	—	2 (16.7)	—
MSSE	1 (3.6)	1 (4.2)	3 (13.6)	—	2 (7.4)	—	—
K. pneumoniae	1 (3.6)	1 (4.2)	1 (4.5)	2 (20.0)	3 (11.1)	2 (16.7)	4 (30.8)
P. aeruginosa	2 (7.1)	2 (8.3)	5 (22.7)	1 (10.0)	5 (18.5)	1 (8.3)	3 (23.1)
A. baumannii	2 (7.1)	1 (4.2)	—	4 (40.0)	1 (3.7)	—	4 (30.8)
Negative sample	6 (21.4)	2 (8.3)	1 (4.5)	—	—	—	—
Total	28 (100.0)	24 (100.0)	22 (100.0)	10 (100.0)	27 (100.0)	12 (100.0)	13 (100.0)

The absolute number of studies in which a particular pathogen was detected and the percentage of all samples are indicated (bottom line).

Table 4

Comparison of occurrence of clinically most significant microorganisms in different periods of follow-up

Pathogen	Period	The 1st week	The 2nd week	The 3rd week	The 4th week	The 2nd month	The 3rd month	The 4th month	Pathogen
E. faecalis →	The 1st week	-	>0.999	>0.999	0.164	0.352	0.209	**0.028**	← *K. pneumoniae*
	The 2nd week	>0.999	-	>0.999	0.201	0.612	0.253	**0.042**	
	The 3rd week	>0.999	>0.999	-	0.224	0.617	0.279	0.052	
	The 4th week	>0.999	>0.999	>0.999	-	0.597	>0.999	0.660	
	The 2nd month	>0.999	>0.999	>0.999	>0.999	-	0.634	0.187	
	The 3rd month	—	—	—	—	—	-	0.645	
	The 4th month	—	—	—	—	—	—	-	
MRSA →	The 1st week	-	>0.999	0.576	—	—	—	—	← *MRSE*
	The 2nd week	>0.999	-	0.600	—	—	—	—	
	The 3rd week	0.621	0.609	-	—	—	—	—	
	The 4th week	—	—	—	-	—	—	—	
	The 2nd month	0.611	0.331	>0.999	—	-	—	—	
	The 3rd month	—	—	—	—	—	-	—	
	The 4th month	—	—	—	—	—	—	-	
MSSA →	The 1st week	-	>0.999	0.301	—	0.611	—	—	← *MSSE*
	The 2nd week	>0.999	-	0.326	—	>0.999	—	—	
	The 3rd week	—	—	-	—	0.641	—	—	
	The 4th week	—	—	—	-	—	—	—	
	The 2nd month	—	—	—	—	-	—	—	
	The 3rd month	0.209	0.253	—	—	—	-	—	
	The 4th month	—	—	—	—	—	—	-	
P. aeruginosa →	The 1st week	-	—	—	—	—	—	—	—
	The 2nd week	>0.999	-	—	—	—	—	—	
	The 3rd week	0.217	0.234	-	—	—	—	—	
	The 4th week	>0.999	>0.999	0.637	-	—	—	—	
	The 2nd month	0.252	0.425	0.737	>0.999	-	—	—	
	The 3rd month	>0.999	>0.999	0.389	>0.999	0.645	-	—	
	The 4th month	0.304	0.321	>0.999	0.604	0.700	0.593	-	

Fisher's two-tailed test was used. The cells of the table indicate the asymptotic significance (p) at the intersections of rows and columns corresponding to the compared terms. For each pathogen, the values are indicated in the corresponding triangle of cells; the triangles are separated from each other by gray cells with single "-" symbols. Statistically significant differences ($p < 0.05$) are highlighted in bold and in yellow.

Conclusion

The microbiological spectrum of IAI pathogens in spinal surgery is one of the most crucial factors to be considered for efficient treatment, also when using negative pressure systems. Monitoring of microorganisms isolated from each individual patient, including evaluation of their possible associations, as well as species composition, is essential for the choice of rational antimicrobial therapy, which is particularly important for the use of NPWT systems due to the duration of treatment. This will help to better understanding the processes occurring at the site of orthopedic spine infection in the future, and therefore to improve the treatment outcomes.

Limitations on the validity of the study

1. The study does not analyze the development factors of infectious complications in spinal surgery (the nature of pathology, surgery duration, absolute and relative blood loss volume, open and minimally invasive surgery, etc.), as detailed in the literature sources and protocols for the control of such complications.

2. The study of the retrospective selective group did not imply the investigation of the microbiological spectrum of pathogens and the efficacy of the use of other treatment techniques for IAI.

3. The issues concerning the analysis of nosocomial flora have been purposely left out of the scope of the study.

The authors believe that the issues reflected in paragraphs 2 and 3 should be the subject of independent research and under a different design.

The study had no sponsors. The authors declare that they have no conflict of interest.

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