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IMPROVING EFFECTIVENESS OF MICROBIOLOGICAL DIAGNOSTICS IN PATIENTS WITH SURGICAL INFECTION OF SOFT TISSUES

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Ключевые слова: *микрофлора, антибиотикорезистентность, биоптаты ран*

Abstract. Improving effectiveness of microbiological diagnostics in patients with surgical infection of soft tissues. Biesiedin O.M., Storubel L.N., Yevtushenko E.V., Biesiedina K.O. *Recently, there has been a steady tendency towards increase in the resistance of microorganisms to antibacterial drugs, which is due to the lack of development of new antibiotics, their uncontrolled administration, total use in animal husbandry, etc. leads to fatal outcomes, unsatisfactory results of treatment, rise in cost of treatment, formation of hospital pan-resistant strains. The increasing ability of microorganisms to cooperate in biofilms, production and improvement of resistance mechanisms in symbiosis are intractable issues in the treatment of patients with surgical infection of soft tissues. In patients with surgical infection of soft tissues, surgical treatment of a purulent focus and antibiotic therapy are fundamental doctrinal, determining the success of treatment.The choice of an antibacterial drug in most cases is based on the results of an antibioticogram. A study of wounds by smear technique and biopsy samples of wound tissues in 81 patients was performed. The quantitative and qualitative composition of wound microflora of patients was evaluated during the study of biopsy samples and standard culture from the wound surface. When examining the biopsy samples, 86 strains were isolated, and 110 – in the material from the wound surface. There was no growth on the wound surface in 11 cases (13.5%), against 20 (almost 25%) in biopsy samples. One species of flora was isolated from 37 wound surfaces (about 46%), biopsy samples of wounds with monoculture – in 61 samples (75%). Associations (two or more) are present in 33 (41%) wound samples, and only in 21 biopsy samples (26%). Thus, the conducted studies confirm the fact that wound surface is more contaminated by foreign microflora than biopsy samples. The data obtained allow us to recommend the method of wound biptates instead of a smear, as more specific and sensitive in patients with surgical infection of soft tissues.*

Реферат. Повышение эффективности микробиологической диагностики у пациентов с хирургической инфекцией мягких тканей. Беседин А.М., Сторубель Л.Н., Евтушенко О.В., Беседина К.А. *В течение последнего времени наблюдается неуклонная тенденция к росту резистентности микроорганизмов к антибактериальным препаратам, что на фоне отсутствия разработки новых антибиотиков, их бесконтрольного назначения, тотального применения их в животноводстве и т.д. приводит к летальным исходам, неудовлетворительным результатам лечения, удорожанию лечения, формированию госпитальных панрезистентных штаммов. Возрастающая способность микроорганизмов кооперироваться в биопленки, выработка и совершенствование механизмов резистентности в симбиозе являються трудноразрешимыми вопросами лечения пациентов с хирургической инфекцией мягких тканей. У пациентов с хирургической инфекцией мягких тканей хирургическая обработка гнойного очага и антибактериальная терапия являются основополагающими доктринальными и определяющими успех лечения направлениями. Выбор антибактериального препарата в большинстве случаев основывается на результатах антибиотикограммы. Проведено исследование ран методом мазка и биоптатов тканей раны у 81 пациента. Оценен количественный и качественный состав микрофлоры ран пациентов при исследования биоптата и стандартного посева с поверхности раны. При исследовании биоптата выделено 86 штаммов, а в материале с поверхности раны – 110. На поверхности раны отсутствует рост в 11 случаях (13,5%), против 20 (почти 25%) в биоптатах. Один вид флоры выделено из 37 раневых поверхностей (около 46%), биоптатов ран с монокультурой – 61 образец (75%). Ассоциации (два и более) имеются в 33 (41%) раневых образцах и только в 21 биоптате (26%). Таким образом, проведенные исследования подтверждают тот факт, что поверхность раны в большей степени*

является контаминированной посторонней микрофлорой, чем биоптат. Полученные данные позволяют рекомендовать метод биоптатов ран вместо мазка как более специфичный и чувствительный у пациентов с хирургической инфекцией мягких тканей.

Worldwide, antimicrobial resistance (AMR) causes 700 000 deaths each year. But the prognosis is much more serious: WHO experts say that if the AMR continues to spread, the number of deaths from antibiotic resistance of pathogens could reach 10 million people a year by 2050 [9, 10].

A similar trend is observed in Ukraine. Thus, the All-Ukrainian Association for Infectious Control and Antibiotic Resistance has analyzed data (2013-2017) of the laboratories of 24 regional hospitals in Ukraine. The results of the study showed that the number of strains of microorganisms resistant to one antibiotic is 70.7%, to antibiotics of 2-3 classes – 37.5%, to 4 or more – 26.3% [5].

Continuous growth of resistance of microorganisms to antibacterial drugs not only leads to deaths and helplessness of doctors, but also adversely affects the time of treatment of patients and increases the cost of therapy [8]. Therefore, laboratory testing to determine the sensitivity of microorganisms is becoming increasingly important.

The aim is to improve the results of treatment of surgical infection of soft tissue in patients undergoing treatment in the department of purulent-septic surgery by introducing a technique for the study of bioptates of wounds for topical personalized antibacterial therapy.

MATERIALS AND METHODS OF RESEARCH

According to the degree of microbial contamination, surgical wounds are divided into four classes: "clean", "conditionally clean", "contaminated", "dirty or infected". The latter includes old traumatic wounds that contain non-viable tissues, and wounds with signs of a pronounced clinical form of purulent-inflammatory infection or with perforation of the internal organs [7].

In 2019, in the laboratory of the Municipal Institution “City Clinical Hospital No 4” of the Dnipro City Council in Dnipro, with the assistance of “Arterium” corporation, bacteriological studies of the wounds of patients with surgical infection of soft tissues, treated in the department of purulent-septic surgery were conducted.

In recommendations of WHO "Basic methods of laboratory research in clinical bacteriology", in addition to the study of purulent exudate, abscess puncture, etc., it is recommended to study small pieces of tissue. The method of quantitative study of biopsy samples has been described in many literary sources: L. Brentano (1965), C.P. Baxter et al. (1974) in modification of laboratory of microbiology and immunology of Institute of Surgery named after A.V. Vishnevsky (Kolcher II et al., 1980). In our clinic, the methodology presented in the monograph "Theory and practice of local treatment of purulent wounds" eds. prof. B.M. Datsenko was used in the study. The studies included parallel bacterial inoculation of tissue bioptates by proprietary methodology and traditional bacterial inoculation from the wound surface [4]. 81 samples of bioptates and material collected from the wound on the swab were included in the analysis.

The study of bioptates includes weighing and homogenization of samples, serial dilutions of material, inoculation of suspension from different dilutions into several nutrient media, subsequent counting, identification of colonies and determination of antibiogram of a possible pathogen.

The obtained material is placed in a sterile container and immediately after sampling with supporting documents (referral to microbiological (bacteriological, virological, parasitological) examination form 204/O) is sent to the laboratory.

In the laboratory, in box settings, bioptate without removing from the standard (with a known mass) capacity, is weighed on electronic scales of the 4th class of accuracy. The mass of the bioptate is recorded and the conversion factor is calculated per 1 g of tissue – K. For example: a bioptate of 0.2 g is delivered; K=1 g : 0.2 g=5.

Later, the weighted bioptate is suspended in isotonic sodium chloride solution at the rate of 1:10, performing a series of ten-fold dilutions to 10^{-8} degree. Inoculation by 0.1 ml of the suspension on the surface of the solid nutrient medium, poured into Petri dishes from dilutions 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}.

To obtain the growth of isolated colonies, the material is carefully rubbed with a spatula over a culture medium for which blood agar is used. Cultures are incubated in a thermostat at 37° C for 18-24 hours. In the absence of growth, the incubation period is extended up to 3 days. In order to accelerate the study of the qualitative composition of microorganisms and improve the diagnosis, inoculation with a loop is carried out on selective differential diagnostic media: Endo, Chistovich and others. Cultures are incubated in a thermostat at 37° C for 18-24 hours. Petri dishes are revised, results are recorded. On Chistovichy medium, in the absence of growth of microorganisms of the genus Staphylococcus, termostatting is continued for up to 48 hours.

Colonies that have grown on the dish are calculated and recalculation per 1 g of tissue is made. For the calculation, those dilutions where colonies on the dish grow isolately are used, with their number not exceeding 300.

The number of microorganisms in 1 g of tissue is calculated by the formula:

N=n x 10 x the degree of dilution x K, where

N is the number of microorganisms per 1 g of tissue bioptate;
n is the number of microorganisms grown on the dish;
10 – conversion per 1 g of suspension;
K – conversion factor of the sample per 1 g of bioptate.

Bacteriological studies and identification of isolated cultures are performed in accordance with national and international standards [2, 3].

Statistical processing was performed using conventional methods [1].

RESULTS AND DISCUSSION

In the study of biopsy, pathogens that are of etiological importance in the development of purulent wound infection were detected in 61 samples (75.3%) of 81. In 20 cases, accounting for only a quarter of the material delivered to the study, the growth of microflora was absent.

Of the colonized microflora of bioptates, one species of microorganisms was isolated in 40 samples from 61 (65.6%). Associations (two or more) are present in a third of positive samples – 21 (34.4%). The total number of isolated microorganisms is 86 strains.

Structure of isolated microorganisms isolated in wound bioptates study

Microorganisms	Gr+ flora							Gr- flora						
	S.aureus	S.haemoliticus	S.epidermidis	E.faecalis	E.faecium	S.pyogenes/ S.pneumoniae	Corynebacteriumspp.	P.aeruginosa	E.coli	P.mirabilis	E.amnigenus	K.pneumoniae	P.penneri	Acinetobacterspp.
Monoculture - 40	17	5	0	6	3	2/0	0	5	1	0	0	0	1	0
Associations - 46	8	5	3	10	4	0/1	2	1	4	4	2	1	0	1
Total - 86	25	10	3	16	7	2/1	2	6	5	4	2	1	1	1

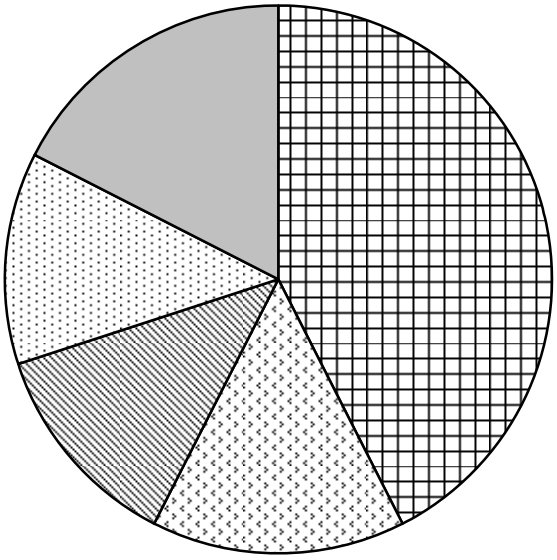
The spectrum of isolated microorganisms by taxonomic affiliation has the following structure. Gr + flora accounted for 77% (66 cultures) of the total number of microorganisms isolated in bioptates and 82.5% (33) of the samples where the strains are found in monoculture. The number of Gr-flora representatives is much less – 23% (20) of the number of all isolated bacteria and 17.5% (7) of the number of microbes isolated in the monoculture.

In the Gram positive group, of all monocultures staphylococci – 55% (22), enterococci – 22.5% (9), streptococci – 5% (*2S.pyogenes*) dominate. *S.aureus* is the leader among staphylococci – 17 cultures, in 5 cases *S.haemoliticus* is isolated. The genus Enterococcus is represented by two species: *E.faecalis* (6) and *E.faecium* (3).

Of all isolated gram-negative cultures, by generic and species characteristics cultures are identified as follows: genus Pseudomonas (*P.aeruginosa* – 6), *Escherichia (E. coli* – 5), *Proteus* (*P.mirabilis* – 4, *P.penneri* – 1), *Enterobacter* (*E.amnigenus* – 2), *Acinetobacter* – 1, *Klebsiella* (*K.pneumoniae* – 1). Of these, in monoculture only 7 strains are isolated: *P.aeruginosa* (5), *E.coli* (1), *P.penneri* (1).

Of all cases found in bioptates of monocultures, 82.5% are related to 4 species of etiologically significant microorganisms in purulent-inflammatory processes: *S.aureus, E.faecalis, P.aeruginosa, S.haemoliticus* (Fig.). A similar trend is observed in the total number of microorganisms. According to data [10], among microorganisms isolated from wounds in medical establishments of Ukraine

in 2017-2016, the following ones prevailed: *Staphylococcus* – 47.1% (48.8%), *E.coli* – 10.7% (*Enterococcus* – 8.6% (8.1%), *P.aeruginosa* – 6.4% (6.5%), *K.pneumoniae* – 6.4% (6.0%), *Enterobacter* – 4.5% (4,4%), *A.baumannii* – 2.5% (3.1%) and others – 13.8% (12.6%).



Main pathogens isolated in monoculture during bioptates study

In bioptates taken from wounds in patients at our clinic, growth was observed in 75.3%, while in sampling the material with a swab – in 86.5%. For comparison, in Ukraine, when studying material taken from wounds, cultures were isolated: 2017 – in 41% of samples, 2016 – in 39.9% [6].

In parallel inoculation of bioptates and smears from the wound surface, a complete coincidence of the study results was observed in 35 patients (43%). More than half of the study results were significantly different – 47 (57%) patients. Thus, in 23% in the absence of growth in the bioptate, from the swab cultures of microorganisms: *S.aureus, P.aeruginosa, S.anginosus, C.albicans, Corynebacteriumspp., S.simulans, S.agalactia, S.epidermidis, P.putida, S.haemoliticus* were isolated. By species spectrum, these microorganisms are representative of normal human microflora, saprophytes and strains wich have signs of hospital ones (*S.aureus, P.aeruginosa*).

In general, when comparing the quantitative and species spectrum of microorganisms isolated in biopsy specimens and smears from swabs, the difference is significant. Thus, in the study of bioptates 86 strains were isolated, and in the material from the wound surface – 110. On the surface of the wound there was no growth in 11 cases (13.5%) against 20 (almost 25%) in bioptates. One type of flora was isolated from 37 wound surfaces (about 46%), herewith 61 samples (75%) – from wound bioptates with monoculture. Associations (two or more) are present in 33 (41%) wound samples and only in 21 bioptates (26%). This indicates that when taking smear from the wound, saprophytic microflora can be inoculated, instead of the flora that causes inflammation, that is, there exists threat of random antibacterial therapy.

Based on the above data, we can conclude that the wound surface is more contaminated with external flora than bioptate. Considering the specificity of the department of purulent-septic surgery, it gives reason to recommend this method for the study of patients with surgical infection of the soft tissues and chronic wounds with the aim of identifying the causative agent of the inflammatory process and prescribing etiotropic treatment, taking into account the antibiograms of isolated cultures.

The antibiograms of the main agents of wound infection isolated from the material of patients of the department of purulent-septic surgery have the following features:

S.aureus is 100% sensitive to vancomycin and linezolid. The sensitivity to erythromycin, oxacillin, clindamycin, levofloxacin is 83%, 80%, 78%, 76%, respectively. Penicillin showed resistance in 44% of strains.

E.faecalis showed sensitivity to ampicillin, vancomycin, linezolid in 100% of cases, instead of ciprofloxacin – only 13%.

S.haemoliticus is 100% sensitive to vancomycin and linezolid; clindamycin – 67%, levofloxacin – 56%, erythromycin, oxacillin – 44%, oxacillin – only 11%.

P.aeruginosa strains differed from other cultures by polyresistance to antibacterial drugs. Of these, only 20% were sensitive to imipenem, amikacin, ciprofloxacin, cefepime, ceftazidime – a third of cultures (33%), meropenem, cefoperazone by 50%, cefoperazone/sulbactam – 60%. The highest level of sensitivity was observed for phosphomycin – 83%.

CONCLUSIONS

1. Tte study shows a quantitative and qualitative difference in the study of bioptates and smears from wound surfaces, this enables to recommend the study of wound bioptates in patients with surgical infection of soft tissues.
2. Due to research performed, we have been able to identify the dominant causative agent of surgical infection and to personalize antibacterial therapy.
3. Topical and personalized etiotropic antibacterial therapy contributed to faster elimination of the pathogen from the wound and preparation of the wound for closure, thereby reducing material costs for antibiotics and the average bed-day. The overall treatment results improved and material costs decreased.
4. In multi-type hospitals, multidisciplinary teams should be fotmed for effective antibacterial therapy of patients with resistant and hospital-acquired infections.

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