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**ANALYSIS OF DYNAMICS OF ANTIBIOTIC RESISTANCE
OF PATHOGENS IN PATIENTS WITH DIABETIC FOOT
SYNDROME UNDERGOING IN-PATIENT TREATMENT**

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Abstract. Persistent ulcers in patients with diabetic foot syndrome in 85% of cases lead to amputation because of secondary infection and growing gangrene that significantly worsen the prognosis. In the course of our study, the data were obtained on the role of the staphylococcus, *Pseudomonas aeruginosa* infections, Enterobacteriaceae isolates, and, in a small percentage of cases, *Candida fungus* in diabetic foot syndrome. It was shown that in case of incomplete eradication of the pathogen the structure of pathogens and its sensitivity to antibiotics undergo changes. In this case, methicillin-resistant staphylococci and *Pseudomonas aeruginosa* are detected. In this paper we discuss the approaches to the choice of antibiotic therapy, subject to the sensitivity of these microorganisms to antibiotic therapy in the surgical department of St. Joseph Belgorod Regional Clinical Hospital. The obtained data state that III-IV generation pseudomonas cephalosporins and fluoroquinolones can be used for empirical therapy. And, in case of staphylococcal infections, - vancomycin.

Keywords: diabetic foot syndrome, infection, antibiotics, antibiotic resistance.

Diabetes mellitus is one of the most common high-cost chronic diseases and the most acute medical and social problem. Over the past decade, the world number of patients with diabetes mellitus (DM) has increased more than 2 times, and reached 387 million people by the end of 2014. According to the prognosis of the International Diabetes Federation, nearly 592 million people will suffer from diabetes by 2035 [1].

One of the late complications of diabetes mellitus is a diabetic foot syndrome, which includes a set of pathological changes on the foot and the lower extremities characterized by the development of venous ulcers, osteo-articular lesions and purulo-necrotic processes in patients with DM on the background of neuropathy and angiopathy. This requires optimization of complex pharmacotherapy [2-10].

Persistent ulcers in 85% of cases lead to amputation because of secondary infection and growing gangrene, which dictates the need for epidemiological studies for identification of patients with diabetic foot syndrome, determination of both the structure of isolated micro-organisms and their sensitivity.

Objectives of the study:

1. To study the structure of pathogens and their sensitivity to antibiotics in patients with diabetic foot syndrome undergoing treatment in the surgical department No.2 of St. Joseph Belgorod Regional Clinical Hospital in 2009-2013.

2. To study the dynamics of changing structure of pathogens and their sensitivity to antibiotics under incomplete eradication of the pathogen and repeated bacteriological study in patients with diabetic foot syndrome undergoing treatment in the surgical department No.2 of St. Joseph Belgorod Regional Clinical Hospital in 2009-2013.

3. To determine the antibacterial chemotherapeutic agents for initial and etiological antibiotic therapy of foot infections in diabetic patients in a surgical department of St. Joseph Belgorod Regional Clinical Hospital.

Materials and methods:

During 2009-2013, there were 109 patients with diabetic foot syndrome treated in the department of purulent surgery of RCH, 61.2% of them were men and 38.8% - women. The average age of patients was 58.9 ± 6.8 years old, the average length of inpatient stay in hospital – 28.3 ± 4.3 bed-days.

93.7% of treated patients suffer from type II diabetes mellitus, the average duration of diabetes was 10.8 ± 4.7 years. 93.7% of patients were in a decompensation phase of DM, and 6.3% of patients – in a phase of subcompensation.

The diagnosis of diabetic foot syndrome and its complications was verified based on a comprehensive survey including data from clinical and laboratory researches, duplex scanning of arteries of the lower extremities, transcutaneous oximetry, and bacteriological tests of necrotic tissue. For determination of clinical forms of diabetic foot syndrome the classification proposed by I.I. Dedov and M.V. Shestakova, 2015, was used [1].

According to the type of course, we revealed neuropathic, ischemic and neuro ischemic variants of the diabetic foot syndrome. The most common was neuropathic course of diabetic foot syndrome – 60% of patients, mixed neuroischemic – 24.2% of patients, and ischemic – in 15.8% of patients.

Material for bacteriological examination was sampled on admission of the patient to the department for treatment, re-sampling of bacteriological discharge - on day 7-10 of inpatient treatment under incomplete eradication of the pathogen. The identification of microorganism strains obtained from the wounds of patients with diabetic foot syndrome being treated in the department of purulent surgery of Saint Joseph Belgorod RCH in 2009-2013. The sensitivity of microorganisms was determined by disk diffusion. Interpretation of the results was performed according to the CLSI (2012) and EUCAST criteria. The calculations were carried out with the “Statistica 10.0” applied statistical software package.

Results and discussion.

During the period of 2009-2013 total 164 strains of necrotic discharge were detected in patients with diabetic foot syndrome (Table 1). Upon analysis of 140 strains (85.4%), isolated from necrotic material at admission of patients to hospital for treatment it was established that the necrotic lesions on patients' foot have predominantly gram-positive microorganisms – 62.2% of cases, gram-negative organisms – 29.3% of cases, and candida fungi – 8.5% of cases.

It was found that the most frequently isolated Gram-positive microorganisms are the genus staphylococci: *S. aureus* (27.1%), and coagulase-negative staphylococci – *S. haemolyticus*, *S. epidermidis* (27.9%). Gram-negative pathogens were mainly represented by *P. aeruginosa* (12.8%), plus microorganisms of family Enterobacteriaceae, *E. coli* (6.1%), *Proteus spp.* (6.7%), and *C. diversus* (2.4%) - Enterobacteriaceae isolates: *E. coli* (6.1%), *Proteus spp.* (6.7%), and *C. diversus* (2.4%). In 2 cases (1.2%) *A. baumannii* was isolated, which is consistent with data from other researchers [11, 12].

In the case when after 7-10 days of empirical antibiotic therapy there remained a purulo-necrotic discharge from foot wound, the patient had to undergo repeated bacteriological examination of wound. Repeated sampling for bacteriological examination isolated 24 strains (14.6%). The findings showed the prevalence of gram-positive microorganisms: *Staphylococcus aureus* – in 8 cases (33.3%), and coagulase-negative staphylococci – in 7 cases (29.2%). Gram-negative bacteria *P. aeruginosa* presented in 4 cases (20.8%). 1 strain of *P. mirabilis* (4.2%) was isolated. Candida fungi were inoculated in 3 cases (12.5%).

Table 1

Frequency of isolated bacterial pathogens isolated from patients with diabetic foot syndrome in 2009-2013*

Pathogens	Number of isolated strains					
	2009	2010	2011	2012	2013	Total
<i>Citrobacter diversus</i>	1		2	1		4
<i>Acinetobacter spp.</i>				1	1	2
<i>Escherichia coli</i>	2	3	3	1	1	10
<i>Proteus spp.</i>	2	2	1	3/1*	2	10/1*
<i>Pseudomonas aeruginosa</i>	2	2	4/2*	5/2*	4/1*	17/5*
<i>Staphylococcus aureus</i>	6/1*	7	13/3*	7/3*	5/1*	38/8*
<i>Staphylococcus spp.</i>	5/1*	9	12/1*	8/3*	5/2*	39/7*
<i>Enterococcus spp.</i>	1	2	3	2	2	10
<i>Candida spp.</i>	2/1*		2/2*	3	3	10/3*
Total						140/24*

*Note: Note: The numerator indicates the number of strains isolated at the first sampling of material for bacteriological study (on hospital admission). The denominator indicates the number of strains isolated at the repeated sampling of purulent necrotic material.

The analysis of antibiotic susceptibility of gram-positive strains recorded increase of resistance to oxacillin in isolated strains of *S. aureus* and coagulase-negative staphylococci during 2009-2013. Figure 1 shows the increase in the number of methicillin-resistant strains of *Staphylococcus aureus* from 20% of cases in 2009 to 50% of cases in 2013, that is less than the level of resistance revealed by domestic authors in the period until 2011 [11]. The same trend was observed among strains of coagulase-negative staphylococci. In 2009-2011 up to 25% of methicillin-resistant strains were isolated, in 2013 – up to 66.6% of resistant strains.

Repeated bacteriological examination revealed a

significantly higher level of resistance to oxacillin in *S. aureus* and coagulase-negative staphylococci than the original, ranging from 66.6% of the cases in 2011-2012 up to 100% in 2009 and 2013. All isolated strains of *Staphylococcus aureus* and coagulase-negative staphylococci in patients with diabetic foot syndrome had sensitivity to vancomycin registered. According to foreign authors, the level of *S. aureus* resistance to oxacillin ranged from 0% of resistant strains isolated from patients undergoing treatment of diabetic foot syndrome in the surgical department of Ouagadougou hospital (Burkina Faso) up to 100% resistance in strains isolated from patients, undergoing treatment at the central hospital in Mexico City [13, 14].

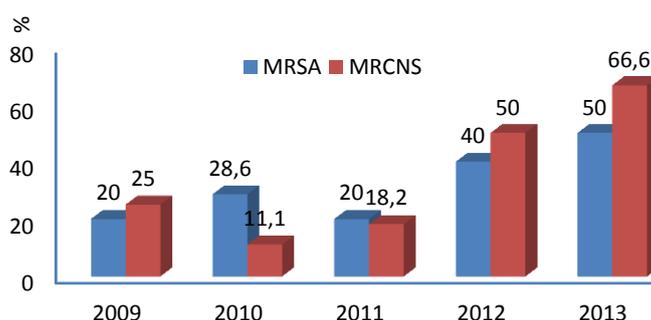


Figure 1. Percentage of isolated methicillin resistant strains of staphylococcus in patients with diabetic foot syndrome on admission in 2009-2013.

Enterococcus spp. was identified in 7.1% of the total number of strains isolated from the purulonecrotic discharge of foot injury taken from patients undergoing treatment in the surgical department No.2 of St. Joseph Belgorod Regional Clinical Hospital,

which is a bit less than the level obtained by US researchers Lipsky B.A. et al., 2012 [14], but exceeds the number of isolated strains according to data by Privolneva V.V., 2011 [11]. The isolated

microorganisms in 60.5% of cases were resistant to

ampicillin at a detectable sensitivity to vancomycin.

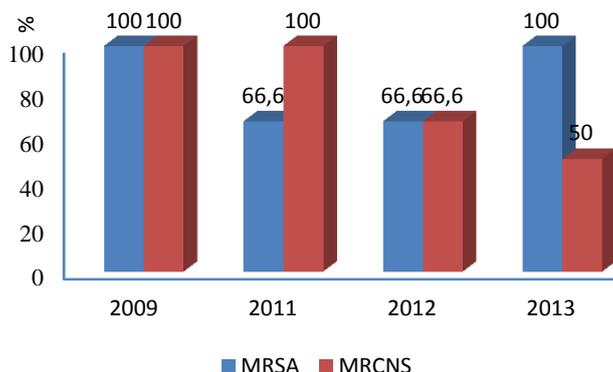


Figure 2. Percentage of isolated methicillin resistant strains of staphylococcus in patients with diabetic foot syndrome on repeated bacteriological examination in 2009-2013.

Studying the *Pseudomonas aeruginosa* sensitivity to antibiotics an increase in resistance level to β -lactams was registered (Table 2). Thus, the resistance to cefepime reduced from 100% in 2009-2011 up to 33.3-66.6% in 2012-2013, at the same time there revealed a low sensitivity to ceftazidime, which was 50% in 2009, and 33.3% in 2011-2013. All isolated strains of *P. aeruginosa* showed sensitivity to meropenem and imipenem, except for one strain isolated in 2012, resistant to meropenem.

We revealed a low sensitivity of *Pseudomonas aeruginosa* to amikacin and gentamicin: from 0% of strains isolated in 2010-2011 up to 50% of strains isolated in 2009 and 2012-2013 were susceptible to amikacin. Sensitivity to gentamicin varied from 33.3% to 66.6% at the preserved sensitivity to netilmicin, which ranged from 66.6% up to 100%. We recorded a low level of sensitivity to fluoroquinolones: *P. aeruginosa* sensitivity to ciprofloxacin ranged from 50% to 66.6%, with the exception of strains isolated in 2011, which showed 100% sensitivity. We revealed a reduction in sensitivity to levofloxacin from 100% in 2009-2010 up to 50-66.6% in 2011-2013. In comparison with the data by Privolnev V.A., 2011, there is an increase in *Pseudomonas aeruginosa* resistance to cephalosporins, fluoroquinolones and aminoglycosides, in contrast to, in particular, netilmicin and less amikacin and gentamicin revealed in the studied period [11].

Repeated bacteriological examination revealed strains of *Pseudomonas aeruginosa* not subjected to a complete eradication and having showed the presence of a reduced sensitivity to cephalosporins, ranging from 0% to 100% at the preserved high sensitivity to imipenem – in 100% of cases. Sensitivity to meropenem was found in 50% of *P. aeruginosa* strains, and variative sensitivity to gentamicin and amikacin was recorded.

In 2010-2011 the amikacin-resistant strains were revealed. In 2009 and 2012-2013 up to 50% of strains staying susceptible to amikacin with continuously reducing sensitivity to fluoroquinolones, which was 100% in 2011, 50% in 2012, 100% for ciprofloxacin and 0% for levofloxacin in 2013. These high variations in sensitivity values are, probably, due to a small number of isolated strains and identify a trend of the developing resistance. The lack of absolute cross-resistance to meropenem and imipenem may be due to the peculiarities of the formation of resistance in *P. aeruginosa*.

Identification of resistant strains of *P. aeruginosa* under incomplete eradication is consistent with Chinese researchers, who showed the relationship between multi-resistant microflora isolated in patients with diabetic foot syndrome, and a higher incidence of amputation in 33.3% of cases as compared with 8.7% of amputations in case of strains with no signs of multi-resistance [17].

Table 2

Susceptibility to *P. aeruginosa* antibiotics isolated from patients with diabetic foot syndrome in 2009-2013

Antibiotics	Number of susceptible strains, %							
	2009 <i>n</i> =2	2010 <i>n</i> =2	2011 <i>n</i> =3	2011 <i>n</i> =1*	2012 <i>n</i> =3	2012 <i>n</i> =2*	2013 <i>n</i> =3	2013 <i>n</i> =1*
Ceftazidime	50	0	33.3	0	33.3	0	33.3	100
Cefepime	100	100	66.6	100	33.3	50	66.6	0
Meropenem	50	100	100	100	66.6	50	100	100
Imipenem	100	100	100	100	100	100	100	100
Ciprofloxacin	50	50	100	100	66.6	50	66.6	0
Levofloxacin	100	100	66.6	0	66.6	50	33.3	0
Amikacin	50	0	0	0	100	50	33.3	100
Netilmicin	100	100	66.6	100	100	100	66.6	100
Gentamicin	50	100	66.6	100	66.6	50	33.3	0

Note: *n* – the number of isolated strains of *P. aeruginosa*, * – the number of strains isolated at the repeated sampling of purulent necrotic material.

The isolated strains of *A. baumannii* (1.4%) had a high level of resistance to different groups of antibacterial drugs. The isolated strains are resistant to penicillins, cephalosporins, fluoroquinolones, aminoglycosides. In 100% of cases, the microorganisms are sensitive only to the carbapenems. The researches of Belgorod Regional Clinical Hospital revealed a higher level of resistance of *A. baumannii* as compared with the data of other researchers [18, 19].

Isolation of non-fermenting bacteria and methicillin-resistant *Staphylococcus* both during admission and during the re-sampling of material for bacteriological study is, probably, due to secondary nosocomial infection, which is associated with admission of patients after both outpatient and inpatient treatment. Identification of *E. coli* and other enterobacteria, perhaps, may indicate infection contamination of the wound under diabetic foot syndrome out of hospital.

The results of the study showed that all isolated strains of Enterobacteriaceae were susceptible on admission to meropenem, imipenem/cilastatin, cephalosporins, which is consistent with the data of domestic researchers [20].

Low activity was observed in gentamicin, netilmicin to Enterobacteriaceae strains isolated in 2009-2011. (33.3-66.6%) at preserved sensitivity to amikacin – 100% sensitivity of the isolated strains among *E. coli* was revealed.

In comparison with the data of other authors, a high sensitivity to amikacin, netilmicin was revealed in strains of *P. mirabilis*. A reduced sensitivity of *P. mirabilis* strains to gentamicin was revealed: strains isolated in 2009-2010 showed resistance in 100% of cases, while in 2011-2013 – only up to 50%.

High sensitivity of Enterobacteriaceae to fluoroquinolones was revealed throughout the entire period of study. Sensitivity to ciprofloxacin and

levofloxacin was 100%, which is consistent with the data of other authors [11].

P. mirabilis strain isolated during the repeated bacteriological examination in 2012 showed its resistance to II-IV generation cephalosporins, amikacin, fluoroquinolones on the background of the preserved sensitivity to carbapenems, netilmicin.

Summary: The main pathogens in case of foot infections on the background of diabetes mellitus are *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *Proteus* spp., *E. coli*.

We have shown an increase in methicillin-resistant strains of *S. aureus*, *S. epidermidis* from 20% - 25% in 2009 up to 50% - 66.6% in 2013.

We revealed an increase in resistance of *P. aeruginosa* to fluoroquinolones and the emergence of strains resistant to carbapenems.

Analysis of bacteriological results showed that there is a change in structure of microview pathogens in diabetic foot under repeated bacteriological examinations. Mostly, the methicillin-resistant strains of *S. aureus*, *S. epidermidis*, *P. aeruginosa* with reduced susceptibility to III-IV generation cephalosporins, aminoglycosides, fluoroquinolones with different resistance variations are identified.

There has been an increase in the number of strains of *Candida* spp. starting from 8.5% of cases at the initial sampling of wound discharge up to 12.5% of cases under repeated bacteriological examination.

Drugs for empirical therapy are III-IV generation pseudomonas cephalosporins and fluoroquinolones. In case of *S. aureus* infections – vancomycin. Perhaps, the use of aminoglycosides after confirming the sensitivity of pathogens thereto in bacteriological examination. The most active agents in case of gram-negative flora are carbapenems, which must be used as a reserve antibiotic therapy after specification of the pathogenic agent.

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