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The bacterial strains isolated from trophic ulcers and their persistence factors

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Abstract

Background: Recently, a particular attention has been drawn to the study of the microbial persistence properties and their correlation with the rate of elimination from the source of infection, as well as the prognosis of the disease progression.
Material and methods: There were examined 44 samples taken from patients with trophic ulcers. The bacteriological examination, as well as tests on determining both the persistence factors and the antibiotic susceptibility of the isolated strains were carried out according to the current method.
Results: There were determined 80 isolated bacterial strains. Two and more strains were isolated in over half of these cases (52.3%). The most commonly involved strains were the genus *Staphylococcus*, followed by *Enterobacter spp*., *Pseudomonas spp., Candida spp.,* and enterococci. Both gram-negative and gram-positive species exhibited a high-level antimicrobial resistance. The study of the persistence factors revealed that the strains isolated in mixed culture showed a higher rate of virulence (1.0-1.5 times higher) compared to isolates in pure culture.
Conclusions: The main bacterial strains isolated from trophic ulcers are the genus *Staphylococcus* and the *Enterobacteriaceae* family. Isolated strains showed higher level of antimicrobial resistance and multiple persistence factors. The study results proved that treatment of trophic ulcers is still a major problem, requiring rational monitoring and management strategies.
Key words: trophic ulcers, microbial spectrum, antibiotic resistance, persistence factors.

Introduction

In recent years there has been a qualitative change of some microbial strains involved in the infectious disease pathology, which tend to increase the incidence of mixed infections caused by potentially virulent gram-negative and gram-positive bacteria and characterized by a marked clinical polymorphism due to a simultaneous exposure of several etiological agents, each of which exhibiting a range of pathogenicity factors [1].

Microorganisms, involved in mixed infections, commonly present antibiotic resistance and a number of pathogenicity factors, such as lecithinase, haemolytic, antilizozyme, DNA-staining, and adherent activity, etc. [2].

Long-term persistence of bacteria within the host organism is due to multiple factors that inactivate the antimicrobial mechanisms of the immune system. Thus, it is highly recommended to study the persistence properties of the microorganisms in purulent infections, since these are responsible for the elimination rate from the site of inflammation, as well as for the prognosis of the disease. It is well known that the bacterial persistent potential is dependent upon the length of pathogenic harboring within the macro-organism, whereas its suppression via drugs may weaken this infectious potential [3-7].

The studies, conducted across different countries, have revealed a range of species isolated from trophic ulcers and their antimicrobial resistance, as well as the incidence of multidrug resistance (MDR) cases, strains of methicillin-resistant *Staphylococcus* spp. (MRS) and extended-spectrum beta-lactamases (ESBL), thus, suggesting that administration of empirical antimicrobial therapy might increase the rate of a treatment failure [8-10].

Treatment of trophic ulcer is a challenging task for clinicians and remains a current and relevant issue [11].

As regarding to the aforementioned, the purpose of the study was to determine the spectrum of bacteria isolated from trophic ulcers, to study the antibiotic susceptibility of the bacterial strains and to determine their hemolytic, lecithinase, anti-lysozyme, and anti-complementary properties, as well as to prove their diagnostic importance in detection of the bacterial targets in order to select the appropriate drug therapy.

Material and methods

Studies were conducted on 80 microbial strains isolated from trophic ulcers. The microbiological investigations, as well as the persistence factors and antibiotic sensitivity assessment of the isolated strains were performed within the microbiological laboratory of the National Agency for Public Health. The bacterial strains were isolated and detected according to the methodology described in "Basic laboratory procedures in clinical bacteriology". Antibiotic susceptibility was tested by Vitek 2 compact and Kirby-Bauer disk diffusion method. Antibiotic susceptibility testing and data interpretation have been standardized, in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for current antibiotic assessment [12]. Antibiotic disk diffusion testing included: cefoxitin (30 mg), erythromycin (15 mg), clindamycin (2 mg), gentamicin (10 mg), cefepime (30 mg), ceftazidime (10 mg) , me-

ropenem (10 mg), aztreonam (30 mg), ciprofloxacin (5 mg), linezolid (10 mg), tetracycline (30 mg), amikacin (30 mg), chloramphenicol (30 mg), rifampicin (5 mg), ampicillin (10 mg). Strains that showed resistance to three or more antibiotic groups were considered polyresistant [13].

The persistence factors were determined in the most common isolates from trophic ulcers. The lecithinase activity was assayed on the egg yolk salt agar, the hemolytic activity on a blood agar plate and the anti-lysozyme and anti-complementary activity we determined according to the method described by Bukharin O. et al. [14-16].

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) reference strains were used for quality control. Statistical data analysis was carried out via EpiInfo 2000.

Ethical Issues

The strains used in this study were obtained from the routine analysis of clinical specimens. Sample collection did not involve direct contact with the patient, thus no consent was required. The study was conducted and approved by the Ethics Committee no. 65 / 12.04.2017 of Nicolae Testemitanu State University of Medicine and Pharmacy from the Republic of Moldova.

Results

The bacteriological study was conducted on 44 samples collected from patients with trophic ulcers. A single bacterial strain was isolated in 36.3% of cases, two and more species – in 52.3% and no strains – in 11.4% of cases.

A total of 80 bacterial species were isolated and identified. The most common strains, isolated from trophic ulcers, were the *Staphylococcus* (predominantly *S. aureus*), then enterobacteria (*Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Serratia* spp., *Escherichia* spp.), non-fermenting bacilli *Pseudomonas spp.,* levuriform fungi of *Candida* type and enterococci. (fig. 1).

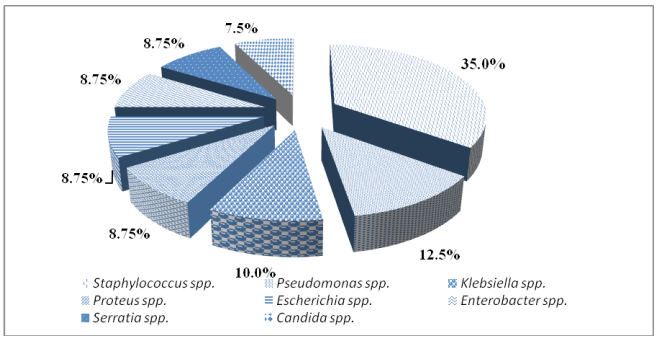

Fig. 1. The etiological spectrum of microorganisms isolated from trophic ulcers.

Among the infections caused by a single strain, the most common was found *Staphylococcus aureus* (43.75%), along with the other isolates such as *Pseudomonas aeruginosa* (18.75%), *Staphylococcus haemolyticus* (12.5%), *Proteus mirabilis* (12.5%), *Klebsiella pneumoniae* and *Enterobacter gergoviae* (6.25%).

S. aureus was determined in 82.6% of mixed infections, whereas 30.4% of cases were associated with *Klebsiella* and *Pseudomonas* species. Two-strain associations were recorded in 52.2% of cases, three-strain in 13.1%, 4 and 5 species were found in 13.1% and 4.3% of cases, respectively.

Staphylococcus spp. strains showed a marked sensitivity to vancomycin (96.4%) and only 1 strain showed intermediate resistance to vancomycin, tetracycline (89.3%) and linezolid (82.1%). Of the 28 tested staphylococcus strains, 13 (46.4%) were methicillin-resistant (MRS). MRS strains were more sensitive to vancomycin (100%), tetracycline (84.6%) and linezolid (76.9%), followed by chloramphenicol (79.2%), whereas a reduced sensitivity was recorded to erythromycin (27.5%) and ciprofloxacin (17.3%). Moreover, the obtained data highlighted a number of strains with multiple antibiotic resistance and only 3 (10.7%) of the 28 strains were sensitive to all the tested antibiotics.

Carbapenems were found to be the most effective antibacterial drugs (86.1%) in treatment of enterobacterial infections; however, the bacteria exhibited a marked resistance to aminoglycosides (> 70%), fluorquinolones and cephalosporins (> 80%).

Furthermore, this study detected 15 extended-spectrum beta-lactamase strains (BLSE), which showed susceptibility to meropenem (86.6%), followed by amikacin (60.0%), gentamicin (53.3%), ceftazidime (26.6%) and ciprofloxacin (13.3%).

The bacterial strains of *Pseudomonas* genus presented susceptibility rates to aminoglycosides (100%) and monobactam drugs (90%) and resistance to fluoroquinolones (100%), carbapenems (90%) and cephalosporins (80%).

Levuriform fungi of the genus *Candida* isolated from trophic ulcers made up 7.5%. In the present study, 66.7% of *Candida* spp. were susceptible to fluconazole, 100% to amphotericin B, 83.3% and 50.0% to voriconazole and itraconazole, respectively.

In the next step of our study, we determined the levels of expression for some persistence factors found in the most common bacterial strains, isolated from trophic ulcers.

The study of the persistence factors of bacteria isolated from trophic ulcers showed a higher- level expression in strains isolated from mixed infections (1.0-1.5 times) compared to those isolated in pure culture.

Lecithinase was among the studied persistence factors. This enzyme destroys lecithin and releases the receptors with which microorganisms interact [16]. Of the total 26 *S. aureus* strains, 24 (92.3%) showed lecithinase activity and 2 (7.6%) strains were inactive.

Hemolysin was determined as another persistent factor, leading to development of chronic infectious disease. Hemolytic activity was detected in 38 (47.5%) bacterial strains isolated from trophic ulcers. A higher level of expression was also recorded in strains isolated from the mixed infections, compared to those isolated in pure culture.

Lysozyme is a carbohydrase that selectively breaks down the glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine and is a component of the peptidoglycan cell wall. If the peptidoglycan network is destroyed, the osmotic pressure between the inside and the outside of the cell is no longer balanced and thus is being destroyed [17]. Therefore, an important strategy of the bacteria is to protect themselves against this enzyme, aiming to survive longer

in the host organism. Of 26 strains of *S. aureus*, 14 (53.8%) strains showed antilysozyme activity and 12 (46.2%) were inactive. The antilysozyme activity was assessed by lysozyme titres in the medium, which revealed that out of 14 Staphylococcus aureus strains, 5 (35.7%) strains showed a lysozyme concentration greater than 10 μg/ml, 6 (42.9%) – a concentration of 5-10 μg/ml and 3 (21.4%) – 5 μg/ml.

The antilysozyme activity of *Enterobacteriaceae* strains was also assessed, showing that of 36 strains, 24 (66.7%) strains exhibited an antilysozyme activity, of which 6 (25.0%) in concentration greater than 10 μg/ml, 8 (33.3%) – in concentration from 5-10 μg / ml, 10 (41.7%) – in concentration of 5 μg/ml and 12 (33.3%) strains did not present any activity ($p < 0.05$).

Another important factor that provides persistence for the microorganisms within the infection site is the ability of the bacterial cells to inactivate the complement system of the macroorganism [16] . The study of the anti-complement activity of the strains isolated in pure culture showed that 62.5% of the strains inactivate the complement and 37.5% of the strains did not present anti-complement activity.

Of the 26 Staphylococcus aureus strains, 24 strains (92.3%) exhibited complementary activity, of which 7 (29.2%) strains inactivated the complement at a concentration of 5 CH50/ml, 3 (12.5%) – at concentration from 5 – 15 CH50/ml and 35 (58.3%) in a concentration greater than 15 CH50/ml. Only two strains did not exhibit anti-complement activity (7.7%).

Anticomplementary activity is a common feature among the bacteria of the *Enterobacteriaceae* family. 34 (94.4%) strains of enterobacteria out of 36 isolates from trophic ulcers showed anti-complementary activity. 1 (2.9%) strain inactivated the complement at a concentration of 5 CH50/ml, 6 (17.6%) at a concentration from 5-15 CH50/ml and 27 (79.4%) strains at a concentration greater than 15 CH50/ml.

The data study of the anti-complementary activities in monocultures compared to isolated cultures in associations (co-culture isolates) showed that the latter are often related to medium and high anti-complementary activity ($p<0.05$).

Conclusions

1. The study of the spectrum of microorganisms isolated from trophic ulcers has revealed the significant role of strains belonging to the genus *Staphylococcus*, followed by enterobacteria, *Pseudomonas* spp., levuriform fungi of the genus *Candida* and streptococci. *Staphylococcus aureus* strain was predominantly isolated in both pure and mixed cultures.

2. Both gram-positive and gram-negative strains isolated from trophic ulcers showed a marked resistance to the antimicrobial drugs tested.

3. The study of the persistence factors confirmed that the strains isolated from trophic ulcers exhibit a range of properties to inactivate the natural resistance factors of the macroorganism.

4. It is essential to understand the pathogenic persistence factors, since it might provide effective targeted therapies for controlling the microbial growth in trophic ulcers.

5. The study results have proved that treatment of trophic ulcers is still a major medical concern, requiring current management strategies.

Conflict of Interest Declaration

There are no known conflicts of interest and financial or non-financial support associated with this publication.

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