THE ROLE AND TYPES OF ANTIBIOTICS, DEPENDING ON THE INJURIES OF SOLDIERS OF IX AND X POLISH MILITARY CONTINGENT IN AFGHANISTAN

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Abstract: The frequency of external wounds sustained by soldiers on the modern battlefield is not declining. In particular, this concerns participants in humanitarian and stabilization missions in the third world countries, almost daily attacked with improvised booby-traps and firing missiles or multi-caliber weapons. The wound infection rate is high, which requires often the empirical use of antibiotics, both in local dressings, as well as the overall dose. The knowledge of the probable causative agent of a wound infection, its theoretical susceptibility to the antibiotic and spectrum antibiotics that are currently available, is a factor which is conditional in the success of treatment. In order to investigate, how the supply of PKW Afghanistan in antibiotics and chemotherapeutic drugs is presented and whether this supply range is suitable for medical problems, we analyzed the consumption of these drugs. Data for the years: 2010–2011 were available. The supply structure was dominated by antibiotics penicillin, cephalosporin, amino glycoside and macrolide. It was stated that each year the supply and consumption of antibiotics increased by 127.9%, with a stable number of serving people and a stable number of sustained injuries. Compared to 2010, in 2011 there were purchases of antibiotics used in severe, complicated infections caused by opportunistic multi-drug-resistant pathogens. This proves that the epidemiological situation was deteriorating on the area of PKW Afghanistan service mission.

Keywords: antibiotics, battlefields, Afghanistan, the consumption of antibiotics, PKW

Injuries sustained by the soldiers on the battlefield almost always are related to the violation of the body shell, and the wounds of combat operations are usually contaminated and infected wounds. The medical reports relating to the operation Iraqi Freedom show that the contemporary battlefield wounds are infected in 48% and wounds resulted from other mechanisms are infected only in 16% (1). The traditional division of surgical wounds into the four degrees of microbiological purity according to the CDC definition qualifies the majority of traumatic wounds as the wounds of III degree and IV degree this is of high or very high risk of infection (2–5). Such wounds should not be quickly closed with primary stitches, but only after the study a primary or delayed stitch should be used (3, 4, 6, 7). Over the years, have been changed the dominant etiological factors of wound infection: during the First World War were dominated Clostridia species, during the Second World War Streptococcus pyogenes and Staphylococcus aureus and rods G (–) (Pseudomonas aeruginosa, Enterobacter species, E. coli, and Klebsiella species) from the Vietnam era (Aronson, Petitta, Mc Lennan, Lindberg, Tong). This change resulted from the consistent observance of the development of surgical wounds, which reduced the risk of hemolytic streptococci and anaerobes, and the use of antibiotics, which effectively reduced the infection caused by pathogens G (+). The experience of military medicine led to the formulation of the doctrine of the early, radical excision of trauma wounds with the removal of infected tissue, impaired by the trauma and the purification of wounds, revision, removal of foreign bodies (6–8). During the current armed conflicts, since the war in Korea, the incidence of gas gangrene is estimated at 0.02–0.08%, with zero mortality (7–12). Antibiotic prophylaxis is the supplement of surgical treatment. Penicillin was used for the first time in a wounded soldier in North Africa in 1942, the entire course of treatment consumed about 40,000 U, and then it was so valuable that the drug was recovered from the urine of the patient (8). Already in 1961, Burke demonstrated in an animal model that the preoperative use of antibiotics significantly reduces the incidence of infectious complications (13, 14).

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Antibiotics used in the prevention of injuries are targeted to the most common microbial flora of the system, area of the body or an organ, their use is therefore an empirical. The body shell is characterized by skin flora micro-staphylococci, including *Staphylococcus aureus* and *epidermidis*. For the nasal cavity, paranasal sinuses are: *Streptococcus* and *Haemophilus influenzae*. For the upper gastrointestinal tract, bile tract, urinary tract, be sure to remember about anaerobe non-spore forming cocci, *Streptococcus pneumoniae*. Other less common causative factors such as *Proteus mirabilis*, *Escherichia coli* and *Engertoccus* are known as the gas phlegmon, now sometimes is referred to as type I necrotizing fasciitis. Most often is caused by not-sporulating anaerobes (such as *Bacteroides type*) or by a mixed flora of Gram positive and Gram negative (anaerobic and aerobic streptococci, staphylococci, Actinomyces species, Enterococcus species, *Pseudomonas aeruginosa*, Enterobacteriaceae such as *Proteus mirabilis*, *Escherichia coli* (17, 25–28). Fungal infections are also described such as fungus *Mucor* types (29, 30).

The treatment of gas gangrene are: crystalline penicillin in combination with metronidazole or aminoglycoside antibiotic, and also lincosamides such as clindamycin, administered intravenously. Semi-synthetic penicillins are also used, such as amoxicillin or piperacillin or ticarcryn in combination with bacterial β-lactamase inhibitors (clavulanic acid), fluoroquinolones, and tetracycline in combination with aminoglycosides (7, 12, 22, 23).

Very similar to gas gangrene clinical course may have non-clostridial gangrene syndrome (17, 25–28). Non-clostridial gangrenous was formerly known as the gas phlegmon, now sometimes is referred to as type I necrotizing fasciitis. Most often is caused by not-sporulating anaerobes (such as *Bacteroides type*) or by a mixed flora of Gram positive and Gram negative (anaerobic and aerobic streptococci, staphylococci, Actinomyces species, Enterococcus species, *Pseudomonas aeruginosa*, Enterobacteriaceae such as *Proteus mirabilis*, *Escherichia coli* (17, 25–28). Fungal infections are also described such as fungus *Mucor* types (29, 30). The treatment of gangrene non-clostridial is based on a rapid and extensive surgical intervention and an empiric, wide-spectral, intravenous, antibiotic therapy. A combination of three antibiotics is proposed, such as penicillin, gentamicin and clindamycin.

Fournier gangrene, multi-bacterial, synergistic gangrene concerns the soft tissue of the perineum, the perianal and male genital organs (17, 31–33). The disease is severe, has an aggressively progressive course and besides the local destruction of soft tissue, causes immediate danger to the life of the patient. Empirical antibiotic therapy should be initiated prior to surgical intervention: should be used a combination of β-lactam antibiotics (crystal penicillin, third generation cephalosporin, ureidopenicillin), aminoglycoside and metronidazole.

Toxic shock syndrome of streptococcal etiology (Streptococcal Toxic Shock Syndrome, STSS), is diagnosed when symptoms of infection are accompanied by hypotension and multiple organ dysfunction syndrome. During the Vietnam War, syndrome and septic shock was the second most common cause of soldiers death, after hemorrhage, coming within 24 hours after the injury. The decisive factor in the occurrence of infection is the virulence of a strain of *Streptococcus pyogenes*. Responsible for the development of infection are M protein-containing strains of M1 and M3, rarely M6, M11, M28 (17, 34–38). The infection spreads rapidly along the routes of lymphatic drainage in the skin and soft tissue and into successive layers of soft tissue. It is crucial to start an antibiotic treatment quickly with clin-
damycin at doses of 600–1200 mg every 8 h i.v. (34–38). Clindamycin has several unique properties: it does not depend on neither the bacterial inoculum nor the growth phase, and inhibits bacterial protein synthesis — streptococcal toxins, including penicillin-binding proteins (PBP), it has longer the post-antibiotic effect than β-lactams. Complementary is a local treatment: the use of dressings impregnated with antiseptics (Octenisept, Hibitan, Betadine) with “half open” method, amended, 1–2× per day.

The problem of external injuries at the Mission of Iraq and Afghanistan is a serious one because it concerns so far a total of about 18,000 participants in the U.S. contingent in the mission of Iraqi Freedom (39). So far, partial data on the Polish case also show the increasing problem of injury to the PKW soldiers in Afghanistan. Because of the nature of warfare, where the injuries are caused by explosions with IED, rocket fire, mortar fire or gunshots, with the widespread use of protective measures at the head and torso, limbs injuries dominate. The wounds are characterized by the presence of traumatic amputations, significant extensiveness and the severity of an injury and the exposure of bones (40). The bacteriology of these wounds determines empirical antibiotic therapy. During Operation Iraqi Freedom samples from the wounds caused by the war taken soon after the injury revealed mainly aerobic bacteria culture (41). Cultures were positive in 50% of cases, and the injury revealed mainly aerobic bacteria culture from the wounds caused by the war taken soon after therapy. During Operation Iraqui Freedom samples of PKW Afghanistan in antibiotics and chemotherapeutics used in the treatment of infected wounds and in prevention of these infections in terms of the current, epidemiological situation in this country.

Experimental

Materials and methods

There was a review of the publication of Polish Military Contingent in Afghanistan covering the period from 01. 01. 2010 to 12. 31. 2011. (changes: VII–X), which is available to the Operational Command of the Armed Forces of Poland. In the period the service performed 10,294 soldiers.

Using the medical records all cases of injuries sustained by soldiers were reported, except for auxiliary services personnel and civilian employees. Injuries were divided into combat and non-combat ones. Combat injuries included all combat situations in which the injury occurred during combat activities: patrol on the ground, repelling attacks at the base or in the field, carrying out activities aiming at actively fighting the enemy. Non-combat injuries included all others. Injuries resulting from trauma were divided into groups according to organ systems /area of the body.

There were also cases in which the injury was the primary cause of medical evacuation grade IV, which was the indirect evidence of the severity of the injury and the occurrence of complications.

Medical data were compared with the structure and the quantity of supply of PKW in antibiotics. The amount of purchased packages were available, the number of tablets / vials in the box, and dose. These data were converted to standard therapy unit...
RESULTS AND DISCUSSION

The structure of the injuries

During the course of mission of PKW, in the analyzed period a total of 380 soldiers suffered various injuries. In these, battle injuries accounted for 87.1% (331 soldiers), a non-combat ones 12.9% (49 soldiers). There were 125 incidents of combat, which resulted in injuries to 331 soldiers. A total of 1037 individual injuries were stated (average 3.13 to
1 soldier, SD = 1.54). Forty one non-combat incidents were reported. They caused damage for 49 soldiers who suffered a total of 70 individual injuries (average 1.43 injuries per 1 individual soldier, SD = 0.71). The average number of injuries resulting from combat operations was significantly higher than in non-combat (p < 0.001).

Multiple injuries occurred in 254, and single in 77 soldiers. Wounds by 291 men (76.6%) resulted in longer than 7 days inability for service, and 190

![Figure 3. The comparison of the quantitative structure of antibiotics purchased for PKW Afghanistan in 2011 and 2010](image)

Table 1. A detailed list of injuries and medical evacuations of the post-traumatic reasons.

<table>
<thead>
<tr>
<th>Location / nature of injury</th>
<th>Combat injury</th>
<th>Non-combat injury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of injuries</td>
<td>Including the cause of evacuation in %</td>
</tr>
<tr>
<td>Limb bone fracture</td>
<td>513</td>
<td>52.5%</td>
</tr>
<tr>
<td>Muscle contusion/rupture</td>
<td>150</td>
<td>38.7%</td>
</tr>
<tr>
<td>Limb amputation/partial amputation</td>
<td>38</td>
<td>100.0%</td>
</tr>
<tr>
<td>Limb vessel rupture</td>
<td>13</td>
<td>92.3%</td>
</tr>
<tr>
<td>Spinal injury</td>
<td>37</td>
<td>81.1%</td>
</tr>
<tr>
<td>Eye injury</td>
<td>35</td>
<td>80.0%</td>
</tr>
<tr>
<td>Acoustic injury/tympanic membrane rupture</td>
<td>23</td>
<td>39.1%</td>
</tr>
<tr>
<td>Facial injuries</td>
<td>43</td>
<td>7.0%</td>
</tr>
<tr>
<td>Bony face injuries</td>
<td>25</td>
<td>68.0%</td>
</tr>
<tr>
<td>Cerebrocranial injuries</td>
<td>36</td>
<td>63.9%</td>
</tr>
<tr>
<td>Chest injuries</td>
<td>21</td>
<td>61.9%</td>
</tr>
<tr>
<td>Abdominal injuries</td>
<td>20</td>
<td>90.0%</td>
</tr>
<tr>
<td>Pelvic injuries</td>
<td>19</td>
<td>100.0%</td>
</tr>
<tr>
<td>Burns</td>
<td>63</td>
<td>19.0%</td>
</tr>
</tbody>
</table>

Interest figures do not total 100% because the majority of soldiers had multiple injuries.
injured (50.0%) ultimately required the evacuation to the country for medical reasons.

During the attacks on the wheel-type IED armored personnel carriers, multiple limb injuries were especially common, including multiple fractures of one bone and/or multiple fractures (often open) within a single limb, accompanied by injuries of one of the body cavity, a pelvis, a spine or a head. Abdominal injuries often proceeded with a damage to the spleen, liver or kidney. Pelvic fractures were associated with the rupture of the bladder and/or urethra. Head injuries were mainly cranio-cerebral injuries, but often accompanied by loss of hearing, balance and vision. In particular, penetrating wounds of the orbit were often observed with the opening of the eye / with a foreign body in the eye. Ear injuries were characterized by acoustic trauma and/or interruption of eardrums. Thoracic injuries occurred most commonly in the form of upper airway burns, pneumothorax, bilateral one, contusions / crushing the lungs, rupture of the great arteries. Back injuries consisted of vertebral compression fractures, spondylolisthesis, fractures of the individual elements of the vertebrae. The most vulnerable were places around cervical, cervico-thoracic and thoraco-lumbar region. Detailed data in this area are shown in Table 1.

The detailed analysis of body injuries which caused the final medical evacuation of 4th grade showed that the most common as a result of the combat events were limb injuries, followed by injuries of the face and facial skeleton injuries, injuries of hearing organs, balance and vision, followed by body cavity injuries and injuries cranio-cerebral. In most cases, the nature of the injuries were numerous, that included more than one area of the body or more than one internal organ. There were also relatively frequent injuries on both sides of the even parts of the body/ even organs.

Table 2. Antibiotics generally used in patients in institutions of PKW Afghanistan.

<table>
<thead>
<tr>
<th>Group</th>
<th>Chemical</th>
<th>Name Number of units purchased in the years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural penicillin</td>
<td>penicillin G</td>
<td>0  550</td>
</tr>
<tr>
<td>Semisynthetic penicillin</td>
<td>amoxicillin, piperacillin, ampicillin</td>
<td>5120 6100</td>
</tr>
<tr>
<td>Associated preparations</td>
<td>amoxicillin with clavulanic acid</td>
<td>8520 15020</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>amikacin, doxycycline, gentamicin, neomycin,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tobramycin, rifampin</td>
<td>307  500 10500 2750</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>linezolid</td>
<td>0   500</td>
</tr>
<tr>
<td>I Generation cephalosporins</td>
<td>cefazolin, cefalexin</td>
<td>1902 450</td>
</tr>
<tr>
<td>II Generation cephalosporins</td>
<td>biofuroxym, cefuroxime</td>
<td>905 2200</td>
</tr>
<tr>
<td>III Generation cephalosporins</td>
<td>ceftazidine, cefoperazone, cefotaxime</td>
<td>5540 2640</td>
</tr>
<tr>
<td>IV Generation cephalosporins</td>
<td>cefepime, ceftriaxone</td>
<td>270 0</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>clindamycin, lincomycin</td>
<td>12000 720</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>meropenem, imipenem</td>
<td>520  0</td>
</tr>
<tr>
<td>Macrolides</td>
<td>roxithromycin, azithromycin, clarithromycin,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rovamycin, erythromycin</td>
<td>2980 2800</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>tigecycline</td>
<td>1100 0</td>
</tr>
<tr>
<td>Quinolones</td>
<td>norfloxacine, nolcin</td>
<td>220 505</td>
</tr>
<tr>
<td>Polymyxins</td>
<td>natamycin</td>
<td>5600 0</td>
</tr>
<tr>
<td>Rifamycins</td>
<td>rifaximin</td>
<td>84 0</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>colistin, miconazole, polymyxin B</td>
<td>400 0</td>
</tr>
<tr>
<td>Other</td>
<td>metronidazole</td>
<td>600 0</td>
</tr>
<tr>
<td></td>
<td>vancomycin</td>
<td>6250 0</td>
</tr>
</tbody>
</table>
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Non-combat events usually resulted in relatively mild injuries, musculo-articular and muscular, the traumatic amputations of individual fingers, the spinal injuries of a spondylolisthesis, or vertebral compression fractures.

An analysis of medical data indirectly shows that 190 wounded suffered serious injuries requiring multidisciplinary treatment and antibiotic prophylaxis.

Analysis of pharmaceutical data

In 2010, it was purchased for PKW Afghanistan 3871 antibiotic packages for topical application, and 28,758 for general application. In 2011 it was purchased 7297 packages for topical application and 34,428 for general application. The increase year to year therefore amounted to 188.5% in relation to topical preparations, 120 % in relation to preparations for systemic use and a total of 127.9%.

In our analysis, reference was made only to the antibiotics of general application, because these, as precisely dosed, were quite closely related to the diagnosis, clinical course and complications. After converting to the standard daily, therapeutic doses, results were obtained suitable for comparative analyses.

The structure of the supply of antibiotics is presented in Table 2. The data in graphic form represent Figures 1–3.

As is clear from the presented analysis, that in the structure of the supply of PKW Afghanistan strongly dominated penicillin antibiotics, from the group of macrolides, and aminoglycosides. The number of doses purchased for soldiers in 2010 and 2011, based on the personal state in those years, did not differ significantly, which can be traced on the number of wounded in each year, although there were in this area seasonal variations (Table 3).

In comparison to 2010, in 2011, the supply of lincosamides increased several times, and in a significant grade also the supply of aminoglycosides (Fig. 3, statistically significant differences at p < 0.000). In addition, in 2011 there were, absent in the supply in 2010, cephalosporin of IV generation of polyenes, carbapenems, polymyxin and rifampycin as well as vancomycin and metronidazole. This proves, that there was the increased demand for antibiotic therapy, which was aimed at combating multiresistant flora, and thus there was a radical change in the epidemiological situation on the area of PKW mission. This is consistent with reports of other authors, according to whom there is a substantial increase in this area, in the number of infections caused by Acinetobacter, Pseudomonas, Klebsiella and Staphylococcus MRSA. In particular, the concern relates to the emergence of tigecycline in a range of supply in 2011. Tigecycline is indicated for the treatment of severe complicated infections caused by strains susceptible to this antibiotic activity, especially complicated skin and soft tissue infections and complicated abdominal infections.

Penicillin still remains first-line drug in PKW Afghanistan, which is consistent with generally accepted recommendations on antibiotic therapy. Given that Streptococcus pyogenes (Group A), Clostridium species and anaerobic Gram (+) cocci are still sensitive to benzyl penicillin, it remains an important drug in the theater of military operations (8). Benzyl penicillin is reserved in the armed forces of the UK to wounds of limbs, soft tissue and muscle, whereas amoxicillin with clavulanic acid in cases of abdominal wounds, and ceftriaxone in combination with metronidazole in the wounds of the head (8).
The microbiological analysis of wounds sustained by soldiers in the attacks of IED type revealed that almost half of the wounds of limbs charged to the participants of the mission in Afghanistan, mainly as a result of IED attacks (49%) showed features of infection and it was allowed to identify microorganisms. The number of microorganisms ranged from 1 to up to 9 in one wound. The vast majority (93%) were G (+) bacteria as saprophytes of the skin. Only three species belong to the G (−) and none was multi-drug resistant.

The only drug resistant pathogen was S. aureus MRSA. Therefore, early prevention is pointless in a broad spectrum antibiotic, aiming mainly against G (−) bacteria such as Pseudomonas aeruginosa and Acinetobacter spp (41).

An antibiotic used empirically in a wounded soldier must have as narrow spectrum as possible and be directed against a probable factor causing infection (8). The single dose of broad-spectrum antibiotic, with a long activity in the body, is recommended as the prevention of infection directly on the battlefield immediately after the injury. Such antibiotic must operate at least during the evacuation from the battlefield (48). Generally, however, the view prevails that the broad spectrum antibiotics should not be used in civilian medicine, initial therapy, as a prophylactic. The hospital always aims at target therapy (7). Antibiotics polypharmacy should be avoided: the application of more than one antibiotic in 24 h does not significantly reduce the risk of sepsis, organ failure or death (49, 50).

The biggest microbiological problem of modern battlefield are multidrug microorganisms: Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter calcoaceticus-baumannii complex (51). Acinetobacter is a common hospital pathogen, attacking trauma patients with wounds and weakened immunity. The problem of the modern antibiotic therapy is the growth of antibiotic resistance, which especially concerns units of hospitals. Numerous hospital pathogens have mechanisms of resistance to many antibiotics, making them a major problem from the clinical point of view, not because of specific virulence factors, but the difficulty in eradicating infections caused by them, the high cost of treatment and potential epidemiological risk to other patients (7). These multi-resistant bacteria included in so-called alert pathogens are mostly Gram (+) cocci – Staphylococcus aureus (MRSA – methicillin resistant, VISA – average sensitive to vancomycin, VRSA – Vancomycin-resistant) staphylococci, CNS, or coagulase – negative example, Staphylococcus epidermidis (MRSE – resistant to vancomycin ), VRE – vancomycin resistant enterococci, Streptococcus pyogenes resistant to erythromycin and multiresistant Gram (−) of the family Enterobacteriaceae, known as ESBL (+), i.e., having a resistance to semisynthetic carpenilins with extended spectrum, and finally the rod G (−) – resistant non-fermentative carbapenems – Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Acinetobacter baumannii (7, 14, 52–54).

The epidemiological situation in a mission in Iraq and Afghanistan is more and more dynamic in recent years. Acinetobacter calcoaceticus-baumannii is often isolated from the war veterans from Iraq in the centers III and IV medical evacuation (53). Acinetobacter bacterium finds favorable conditions during emergencies, disasters and calamities: it was often identified with the wounded after the earthquakes in Turkey, after the tsunami, floods, and as a perpetrator of pneumonia in Lebanon. It colonizes the hospitals, especially air conditioners and fans (51). The microbiological data of hospitals in Iraq, Kuwait and Afghanistan, in turn show that Acinetobacter is developing in the wounds at a later date, in the later stages of medical evacuation, and at stages I and II the most common are mainly cocci (55): Staphylococcus aureus, coagulase-negative (up to 34% of isolates), S. aureus (26%), including MRSA, and Streptococcus sp (11%). The others are: Klebsiella pneumoniae (13%), Acinetobacter calcoaceticus-baumannii (11%), Pseudomonas aeruginosa (10%).

Both G (−) and G (+) bacteria showed plural resistance (41, 55, 56). The development of infections caused by Acinetobacter species and other opportunistic bacteria and the disclosure of clinical symptoms takes time, because most of the victims of IED explosions with the fractures of limbs had at the time of the admission to hospitals the characteristics of wound infection by A. calcoaceticus-baumannii complex, Enterobacter spp. and P. aeruginosa, even without signs of infection at the wound. Secondary streptococcal infections delay healing or amputations (57).

Most isolates of Acinetobacter baumannii in Operation Iraqi Freedom and Enduring Freedom demonstrated a sensitivity to colistin, minocycline, and polymyxin B. In the years: 2003–2005, resistance to imipenem was built up, reaching 87% of resistant strains (57). It is colistin that is recommended as an extremely effective against Acinetobacter baumannii (58). Tigecycline and gycycline have a wide spectrum and an application in
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Skin infections, soft tissue and abdominal cavity. Daptomycin, lipopeptide, with low risk of induction of resistance, is indicated for infections of the skin. Linezolid, the first marketed synthetic oxazolidinone shows high activity against multi-resistant bacteria staining positively in the method of Gram (59).

The problem in explosions in closed, combat wagons are extensive burns, including the respiratory tract. In about 10% of patients with burn wounds comes to sepsis, caused by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus-baumannii* complex, and *Staphylococcus aureus*. Bacteremia caused by *Klebsiella pneumoniae* is the most difficult process, fraught with the greatest risk of mortality (60).

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