SKIN AND SOFT TISSUE INFECTIONS: MICROBIOLOGY AND EVIDENCE BASED ANTIBIOTIC THERAPY

INFECȚII CUTANATE ȘI ALE ȚESUTULUI SUBCUTANAT: MICROBIOLOGIE ȘI TERAPIE ANTIBIOTICĂ BAZATĂ PE DOVEZI

Laura Maria Lucia Papagheorghe1, Mihai Lupu1, Andra Georgiana Pehoiu1, Vlad Mihai Voiculescu1, Raluca Papagheorghe2

1 Department of Dermatology, Elias University Emergency Hospital, Bucharest, Romania
2 Microbiology Department, Central Laboratory, Colțea Clinical Hospital, Bucharest, Romania

Corresponding author:
Laura Papagheorghe, 17 Mărăşti street, sector 1, Bucharest
Phone 021/316 1600 - 190/224, fax: 021/317 3052
E-mail: laura.papagheorghe@gmail.com

Abstract

Skin and soft tissue infections are a frequent reason to address the dermatologist's office. Their evolution depends both on the patients' comorbidities and on the pathogenicity of the infecting microorganism. The antibiotic therapy is the core of the treatment. Its success is the result of the co-operation between clinician and microbiologist. Well tailored initial therapy, guided by local flora and the Gram smear data may enhance success and reduce complications.

Keywords: SSTIs, microorganisms, virulence factors, antibiotic therapy

Rezumat

Infectiile cutanate și ale țesutului subcutanat sunt un motiv frecvent de adresare a pacienților la cabinetul medicului dermatolog. Evoluția acestor infecții depinde atât de comorbiditățile pacienților cât și de patogenicitatea agentului infecțios. Terapia antibiotică este esența tratamentului. Succesul acesteia este rezultatul cooperării dintre clinician și microbiolog. O terapie inițială adaptată corect, ghidată de flora locală și colorația Gram, poate crește șansele de succes terapeutic și poate reduce complicațiile.
Epidemiology

Skin and soft tissue infections (SSTIs) are a border pathology. Their management may yield medical co-operation among several specialties. In USA 14 million patients address for SSTIs yearly and their number is increasing, both in inpatient (29% from 2000 to 2004) and outpatient settings, as well as in the emergency room (ER). [1] SSTIs are a burden to the healthcare systems; they are incompletely studied and necessary care resources are yet unknown. Rare epidemiological studies [2-5] show the increasing prevalence and augmented interest of the scientific medical community towards this pathology.

Etiology and pathogenicity

SSTIs have different degrees of severity, ranging from mild to life-threatening; they present with or without complications, but the common feature is the need to diagnose the infecting microorganism(s). The most common are staphylococci, with Staphylococcus aureus being the main infective agent. Pseudomonas aeruginosa is the second most frequent pathogen. Gram negative bacilli belonging to the genus Enterobactericeae are third most common, together with enterococci and other streptococci. Coagulase negative staphylococci (CoNSs) are controversial as pathogens. [6, 7]

Pathogenicity of S. aureus and CoNS

Staphylococcus spp. (Figure 1) colonize the anterior nose of mammals and may be a contaminant as well as a pathogen in SSTIs. However, when obtained in a culture from a SSI, it is usually a pathogen. Moreover, patients tend to develop infections with own flora, outside epidemiologically confirmed nosocomial infections.[8, 9] 20-30% of normal people shelter MRSA - meticillin resistant S. aureus strains. [10] Six categories of factors contribute to infection and to evasion of host defense mechanisms; each is encoded by specific genetic elements. The pathogenicity of different strains varies according to the clonal type or to the genetic expression of virulence factors; little is known of their expression during infection.[11]

1. Adhesion factors:

Surface proteins adhere to various cellular structures, thus enabling the microorganism to enter the endothelial cell. It initiates endovascular infections and may persist in the endothelial cell. Thus it may account for chronic or recurrent infections with the same strain. Adherence molecules (MSCRAMM-microbial surface components recognizing adhesive matrix molecules) enable staphylococci to bind to vascular devices. Protein A (Spa) belongs to the MSCRAMM family because it binds to the von Willebrand factor, a large glycoprotein that mediates platelet adhesion to the damaged endothelial cell. Spa displays several additional properties: it interferes with immunoglobulin-mediated opsonization, it mimics a B-cell superantigen and it binds to TNF-R1. These proteins also adhere to vascular catheters and prosthetic valves. The binding to the endothelial cell enables a phagocytosis-like internalization process that favors the intracellular persistence of the staphylococci [12, 13]

2. Persistence factors:

Polysaccharide production, intercellular adhesion, small-colony variants, and intracellular persistence contribute to biofilm formation. Cells belonging to the biofilm community have a different metabolism and a different antibiotic susceptibility from the planktonic cells recovered from cultures. Therefore, antibiotic therapy (ATB) schemes remain ineffective in patients having intracorporeal devices with biofilm.[14, 15]

3. Evading/destroying host defenses:

Leukocidins (e.g., Panton- Valentine (PVL) and g-toxin), capsular polysaccharides (e.g. protein A) Invasive skin infections and necrotizing pneumonia produced by community acquired meticillin resistant S. aureus (CA-MRSA) strains are often associated with PVL, abscesses are associated with capsular polysaccharides.

4. Tissue invasion/penetration enzymes: 

proteases, lipases, nucleases, hyaluronate lyase, phos-
**Phospholipase C** and metalloproteases (elastase) are encoded by separate genetic determinants (V8, hysA, hla, plc, sepA) causing tissue destruction and metastatic infections.  

5. **Toxin-mediated disease and/or sepsis:**

Enterotoxins, toxic shock syndrome toxin-1, exfoliative toxins A and B, a-toxin, peptidoglycan, and lipoteichoic acid, food poisoning, toxic shock syndrome, scalded skin syndrome, bullous impetigo, and sepsis syndrome.

6. **With poorly defined role in virulence:** coagulase and bacteriocin

The pathogenicity of the *S. epidermidis* (CoNS) strains is related to the resistance to antimicrobial agents, to the production of invasins and the formation of biofilm. The resistance to oxacillin is high, almost 80% in some studies. Lipase activity has contributed to the ability of invading the dermal-epidermal tissue; the proteolytic activity plays a proven role in tissue destruction and the inflammatory response of the host. Adhesins which facilitate the formation of biofilms have also been related to the proliferation of *S. epidermidis* on the surface of the intravascular catheters and implants and have been involved in tissue invasion.

Methicillin resistance in *Staphylococci* strains renders supplemental difficulty to therapeutic ATB schemes. MRSA are more virulent than MSSA. Methicillin resistant CoNS act as reservoir of resistance determinants.  

**Pathogenicity of Gram negative bacilli (Enterobactericeae and *P. aeruginosa*)**

The common pathogenic factor is the endotoxin, from the bacterial cell wall. It is a deadly structure because it triggers septic shock. Its pathogenic activity is identical, regardless of the bacterial origin. It consists essentially of the injury of the vascular endothelium and the activation of cellular and humoral immune factors, of which the by-passing complement activation plays a key role. The lipopolysaccharide (LPS) in the endotoxin structure attaches to the circulating proteins (LPS-binding protein), and the formed complex is bound to a specific receptor (CD14) on monocytes, macrophages and neutrophils. A lipid component (Lipid A) and a common polysaccharide complex (core) represent the toxic part of the endotoxin macromolecule which together with the specific polysaccharides determines the structure of O antigen (somatic), a basic constituent of the wall of the Gram-negative bacteria (both bacilli and cocci). The stimulation of CD14 lymphocytes (even with low doses of 10 pg/mL) generates intracellular signals via a related receptor, "Toll-like receptor", protein 4 (TLR-4), leading to the activation of the mononuclears and the production of a strong cytokine: IL-1 and tumor necrosis factor (TNF). They act on the endothelial cell and cause various effects, including decreasing synthesis of anticoagulant factors. The effects of cytokines are amplified by the activity of TLR-4 on the endothelial cells. The activity of TLR triggers actions of the complement system by means of by-pass activation.  

In addition to the endotoxin factor, the exotoxins, capsular polysaccharides, cilia can add to the virulence activation in some species, the latter playing a role in the attachment to and colonization of the host cell. *P. aeruginosa* (Figure 2) is a nosocomial pathogen, unlike the other agents discussed.

The first step in the *P. aeruginosa* infection is the colonization. Normally, 3-6% of the people can be *P. aeruginosa* carriers; in hospitalized patients the carrying ratio increases to 50%. In *P. aeruginosa* the virulence usually occurs after a substantial break of the first-line defense barriers (trauma, surgery, burns, intracorporeal systems implanting), imbalances of the normal flora of the mucous membranes as a result of the broad-spectrum antibiotic treatment or of the impairment of immunological mechanisms (in neutropenia).  

The exotoxic factors secreted by *P. aeruginosa* can add to the aggressive virulence equipment.

### Table 1. Examples of broad spectrum ATBs and the possible permissive effect

<table>
<thead>
<tr>
<th>Broad spectrum ATB</th>
<th>Microorganisms able to survive by permissive effect</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporin’s and carbapenems</td>
<td>Enterococci</td>
<td>Enterococci are naturally resistant to cephalosporins</td>
</tr>
<tr>
<td>Second and third generation cephalosporins</td>
<td>Extended Spectrum betalactamases (ESBL) producing Gram () bacilli</td>
<td>Resistant to all betalactams, except carbapenems and betalactamase inhibitors (clavulanic acid, sulbactam, tazobactam )</td>
</tr>
<tr>
<td>Third generation fluoroquinolones All anti Pseudomonas antibiotics (Ex: anti pseudomonas carbapenem; piperacillin, ticarcillin, colistin)</td>
<td>Multidrug resistant <em>P. aeruginosa</em> (MDR-PA) and Acinetobacter spp.</td>
<td>Currently, many species are MDR, susceptible only to colistin. This is the last resource antibiotic against Gram () bacilli.</td>
</tr>
<tr>
<td>Glicopeptides and oxazolidinones</td>
<td>Vancomycine resistant enterococci (VRE) Resistant <em>S. aureus</em> (Vancomycine inhibitory <em>S. aureus</em>) and CoNS</td>
<td>Although ATB options are available against</td>
</tr>
<tr>
<td>All ATBs</td>
<td>Clostridium difficile</td>
<td>Clinical manifestations of <em>C. difficile</em> infection require infectious diseases specialist’s opinion.</td>
</tr>
</tbody>
</table>

Skin and soft tissue infections: microbiology and evidence based antibiotic therapy
Exotoxin A is produced by most clinical isolates; it determines the inhibition of protein synthesis. It causes local tissue damage, invasiveness and could cause even immunosuppression (inhibits the response of the macrophages).

Proteases play a major role in producing infections. The proteolytic capacity of P. aeruginosa is a major determinant of virulence in acute infections. Elasim, a major compound of blood vessels, is destroyed.

LPS (has the same role as in other Gram-negative bacilli) causes sepsis.

Leukocidin inhibits the lymphocytes and neutrophils function.

Pyocyanins inhibit other bacteria, cause oxidative damage to the tissues, particularly the oxygenated tissues, such as the skin and lung.

Capsule production: high molecular weight polysaccharides, recovered in strains isolated from clinical samples. This is one of the major virulence factors meant to evade clearance from an infectious site.

The capsule provides bacteria with protection from the host immune response as well as antibiotics, by not allowing opsonizing antibodies to be recognized by phagocytic host defense cells. This enhances inflammatory response in their effort to clear the microorganism.[22] Although multi-drug resistance (MDR) is not a virulence factor per se, it “protects” bacterial colonization, indirectly contributing to the activation of the pathogenesis of Gram-negative bacilli. P. aeruginosa acts as reservoir of resistance determinants for other Gram negative bacilli. The association with an immunosuppressant background often creates catastrophic circumstances.[23]

Pathogenesis of enterococci spp infections

Enterococci (Figure 3) are a group with reduced or incompletely understood intrinsic virulence.

Several virulence factors have been reported:

- The AS substance (aggregation substance) responsible for the adherence, internalization and intracellular survival.

- The biofilm-producing ESP substance, especially in the strains of the urinary tract infections.

- The pilis responsible for the adherence and biofilm production.

Gelatinase (GelE) is a zinc-dependent extracellular metalloendopeptidase secreted by E. faecalis which has characteristics similar to those of elastase in P. aeruginosa; GelE can hydrolyze gelatin, casein, hemoglobin and other bioactive peptides, which makes it a potential virulence factor in enterococci.

Cytolysin has hemolytic activity on the human red blood cells and bactericidal activity on other Gram-positive bacteria.

The pathogenicity of the enterococci is especially connected to the multi-drug resistance expression. The resistance to vancomycin is the hardest situation to overcome during treatment.[24]

Pathogenicity of β-hemolytic streptococci

The most important pathogen in this group is Streptococcus pyogenes or Group A streptococcus (SHA) (Figure 4).

It produces necrotizing fasciitis, abscesses as well as immune diseases (glomerulonephritis, cardiac and neurologic long term complications). Group B, C, G, and F are less virulent; they accompany S. aureus in most lesions. Pathogenic factors of SHA are better described, as a result of the severe evolution of infections.

It produces an extracellular invasion by braking down the barriers of a tissue and disseminates within the host while remaining outside of host cells. SHA produces enzymes with citolytic effect that cleaves proteoglycans in connective tissue, breaks down fibrin clots, degrades accumulated host oils, and digests released RNA and DNA.

Haemolysins (which punch holes in host cells) expressed by these species destroys erythrocytes and other cell types as well and may also contribute to their spread in host tissues.

Extracellular invasion allows these pathogens access to niches in tissues where they are able to
proliferate, disseminate to other sites in the body, express toxins, and initiate inflammatory responses.\textsuperscript{[25]}

**Microbiology findings**

**Sample collection**

Clinical manifestations of infection such as local redness, lymphangitic spread, swelling rapid progression of lesions and bullae, crepitus, are signs of increased severity. Systemic inflammatory response (SIRS): temperature >38°C or 24 breaths per minute, tachycardia >90 beats per minute, or white blood cell count >12 000 associated with C reactive protein >5 ng/dl and positive procalcitonin test may yield for an invasive infection. In these cases, blood cultures (BC) and biochemical evaluation are needed. BC are necessary when there are manifestations of lymphedema, immune deficiency, fever, pain out of proportion to the findings on examination, tachycardia, or hypotension, especially in patients with infections involving specific anatomic sites, such as the mouth and eyes.

Whenever lesions require incision and drainage or with spontaneously draining purulent fluid, carbuncles, furuncles, boils, cellulitis with purulent drainage, chronic ulcer, and deep wounds, the preferred method is pusz aspiration.

The main difficulty in the microbiology of SSTIs is the ability to differentiate a contaminant from an infective agent. Communication with the laboratory regarding the most appropriate sampling mode, a smear from aspirated puss and/or clinical data are of great use.

**Gram Stain and cultures**

Easy to perform and cost effective, these investigations are recommended for impetigo and eczema. They are intended to help identify SHA, to estimate the Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score\textsuperscript{[26]} and evaluate glomerulonephritis risk.

Furthermore, cultures assess the presence of Staphylococcus and aim to determine MRSA. Blood cultures (BC) are recommended (strong, moderate), and cultures and microscopic examination of cutaneous aspirates, biopsies, or swabs should be considered in patients with malignancy on chemotherapy, neutropenia, severe cell-mediated immunodeficiency, immersion injuries, and animal bites (weak, moderate).

In pyomyositis BC are recommended (strong, moderate), along with abscess cultures.\textsuperscript{[27]}

In erysipelas and cellulites cultures are not recommended. Microbiological report of a gram smear should be able to communicate the presumable infective agent, staphylococcus spp, streptococci Gram (+) bacilli (with differentiation of Enterobacteriaceae from other species), or fungi. It should describe the presence of leukocytes and their report with the microorganisms (intra or extracellular). In figure 1 we show one aspect of staphylococcal infection.

**Antibiotic treatment**

Antibiotic therapy is dictated by the patient’s clinical appearance. The empiric therapy may be guided by international guidelines.\textsuperscript{[28]} The initial therapy is based on local data of antibiotic susceptibility of circulating microorganisms (annual cumulative reports of SSTI pathogens and their susceptibility). Both these therapeutic approaches are life-saving in many situations. However, the latter spares last resource ATBs, by addressing the local pathology. The more appropriate the ATB therapy, the best therapeutic success.

After the complete microbiological report is received, de-escalation therapy is applicable. Thus, the patients will receive a more appropriate agent, with the narrowest activity specter.

The nature of ATB treatment prior to the sample collection is useful information. It enables the rationale of the permissive effect.\textsuperscript{[29]}

Treatment with a broad spectrum ATB (i.e. following the guidelines), kills all susceptible microorganisms. This will enable the survival and multiplication of naturally resistant species. Pathogenic or not, will be able to colonize necrotic tissue and/or produce infections in immunocompromised patients. In table 1 we show some examples of broad spectrum ATBs and the possible permissive effect.

The need of ATB therapy is the dermatologist’s option.

However, many specialists tend to give too much credit to this therapy. Sometimes it is overused unnecessarily in SSTIs contributing to MDR pathogens. Low resource settings, scarce microbiological availability, high prevalence of SSTIs and insufficient study and communication among specialists are only some reasons to overuse ATBs.

**Conclusions**

The algorithm of diagnosis and treatment of SSTIs differs very little from that of other infections. This paper enhances three important aspects of dermatology in the era of multidrug resistant microorganisms:

- the patient outcome may be improved as a result of effective communication between the microbiology laboratory and the dermatologist;
- local studies of SSTIs pathogens and their annual susceptibility contributes both to the therapeutic success and to the practice of sparing last resource ATBs. Preservation of skin microbiome is an important factor of local homeostasis.
- molecular biology findings explain the gravity of persistent and recurrent infections.
Bibliography

1. Ragun, S. Skin and soft tissue infections: classifying and treating a spectrum. Cleveland cl inic journal of medicine. 79(1)


