© Military Pharmacy and Medicine • 2012 • 2 • 32 – 40

Toxicology

Microbes indicators of cosmetic preservation efficiency. Part I – *Pseudomonas aeruginosa*

Jerzy Mierzejewski [1], Agnieszka Woźniak Kosek [2]

[1] Emeritus Professor at the Military Institute of Hygiene and Epidemiology, Pulawy, Poland. Professor at Kazimierz Pułaski Technical University of Radom, Faculty of Materials Science, Technology and Design, Chair of Chemistry, Radom, Poland
[2] Department of Influenza Virus Research, National Influenza Center. National Institute of Public Health–National Institute of Hygiene, Warsaw, Poland

Author's address:
Agnieszka Woźniak Kosek, Department of Influenza Virus Research, National Influenza Center, National Institute of Public Health–National Institute of Hygiene, ul. Chocimska 24, 00-791 Warsaw, Poland; phone: (+48) 225421274, e–mail: akosek@pzh.gov.pl

Received: 2012.05.24 • **Accepted:** 2012.06.08 • **Published:** 2012.06.28

Summary:

The bacterium *P. aeruginosa* is used, among others, as an indicator of the official assessment of the effectiveness of cosmetics preservation. In the hereby study the general characteristics, morphology and culture, diagnosis of infections caused by P. aeruginosa, the role of *P. aeruginosa* in the environment, Pathogenicity of *P. aeruginosa*, *sensitivity and resistance to antibiotics* and current interest with *P. aeruginosa* in cosmetic microbiology were discussed.
The aim of the study is to strengthen the belief of producers and users of cosmetics to the validity of the selection of indicator organisms for assessing the effectiveness of added preservatives.

Key words: *Pseudomonas aeruginosa*, cosmetics, pollution, maintenance of biofilm, antibiotics.

Introduction

In cosmetics, as in other products made from organic material, there may be microbial contamination of various types. Apart from the same raw materials, the source of pollution can be: the environment of production facilities, apparatus and equipment, and improper hygienic method of manufacture and packaging cosmetic. Microbial enzymes that could potentially contaminate the product, causing decomposition of organic components which contribute to lower values of cosmetics. Environmental micro flora presented in air or settled, and taking the form of a biofilm, often causes contamination of the product. Saprophytic species (usually non-pathogenic) bacteria and microscopic fungi occur in the product.

According to current regulations in order to prevent spoilage of cosmetic products, producers are required to determine the minimal but effective concentrations of preservatives. For this purpose, they use the force method called controlled environmental microbial contamination.

In this contaminating micro flora microorganisms of hygienic importance may be found. For this reason, the legislation require to carry out similar pollution-controlled studies using representative

species of conditionally pathogenic microorganisms: *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* [1].

The hereby study consists of three parts which characterizes the abovementioned micro-organisms with particular reference to pathogenic properties and it aims to strengthen the belief of producers and potential users of cosmetics for the validity of the selection of indicator organisms for assessing the effectiveness of added preservatives.

Part I of the study concerns the development of bacterial indicator *P. aeruginosa* that represents a lot of environmental mesophilic and aerobic gram-negative bacilli.

General characteristics, morphology, and culture of *P. aeruginosa*

This species, formerly known as *P. pyocyanea*, is widespread in nature. It occurs in damp areas (soil, water, sewage) [2]. Among the species of the family *Pseudomonacea* is a species responsible for most infections in humans. It can cause opportunistic infections in patients with immune deficiency, even sepsis [3]. This organism can also be pathogenic to animals and plants [4].

Pseudomonaceae are straight or slightly curved gram-negative bacilli, with dimensions of 0.5x1.5 micron, covered with cilia, decomposing glucose. They have very low nutrient requirements, can use non-conventional carbon sources, and are widespread in nature. These bacilli have a significant part in the biodegradation of various chemical compounds polluting the environment.

They are common in hospitals, often in places with high humidity (siphons of sinks, humidifiers of ventilators, drug and disinfectants solutions and in distilled water, where they use even trace amounts of organic substrates to proliferate.

Pseudomonaceae family bacilli showing the following common features:

1) They grow at temperatures from 5 to 45^{0}C;
2) They are moveable, endowed with cilia arranged pole or on the entire surface;
3) They typically produce cytochrome oxidase. *They are capable of growing under aerobic and anaerobic conditions*;
4) Most tribes produce pioverdin pigments and the other ones different colors: pyocyanin, pyorubin, melanin.

There are several divisions of the *Pseudomonadaceae* family bacilli on serological groups and serotypes, biochemical divisions and divisions on different enzymatic profiles.

Genotypic divisions are more accurate, using restriction profiles, ribotyping, caryotyping and profiling by amplification production. [4].

The discussed species of *P. aeruginosa* under aerobic conditions generates energy by organic substrates fosforization: carbohydrates, acids, aromatic compounds (14). However, in the absence of oxygen *Pseudomonas aruginosa* has denitrifying ability due to having dissimilation nitrate reductase [3]. This enzyme is located in the cytoplasmic membrane, and its synthesis is inhibited by oxygen. It should be distinguished from assimilative nitrate reductase – cytoplasmic enzyme, which allows bacteria to use nitrate as a nitrogen source [5].

P. aeruginosa is a bacterium which was developed at the earliest map of chromosomes [3]. The size of the chromosome of *P. aeruginosa* measured in millions of base pairs (MBP) is 5.36 [2] with the number of 7384 genes [3].

In the world of microorganisms, resulting in lisogenic conversion, many plasmids encode proteins secreted extracellularly or short peptides, which kill or inhibit the growth of related bacteria. These substances called bacteriocins and produced by *P. aeruginosa* are known as pyocin [5].

Diagnosis of infections caused by *P. aeruginosa*

The routine procedure of the drawn material for testing is inoculated on selective medium. Nowadays the substrate with the following selectivity factors is used: 0.02% cetrymid (bromide of ethyl trimethyl ammonium) and 15 mg/l nalidixic acid.

Planted sample is incubated at 37^{0}C for 24-48 hours.

P. aeruginosa forms on substrates gray, large colonies with a diameter of 2 mm, slightly dull (sometimes metallic) with irregular edges and carried over middle. Breeding on agar media is accompanied by a pleasant scent of jasmine.

Pigment production

Pigments produced by these microorganisms are secondary metabolites, i.e. they do not belong to the compounds that are present in all organisms. It is obvious from their structure that they come from a normal cell metabolites or its building subunits. Some pigments have antibiotic activity, and many microorganisms producing pigments produce antibiotics as well. It can be assumed with high probability that the microorganisms that produce pigments will also produce antibiotics and other active substances [3].

Production of diffusing pigments into the medium is quite common in aerobic gram-negative bacilli of low-maintenance nutrition. This is an important feature of identity, but is not characteristic of the species. Produced pyocyanin from light green to dark blue shade, is soluble in water and chloroform.

Pyocyanin is also produced on a selective medium containing cetrymid. This pigment is only produced by strains of *P. aeruginosa*, but except from pyocyanin they also produce other fluorescent pigments under UV light: yellow-green fluorescein and yellow pioverdin, brown biomelanine and red piorubrin. The best conditions for pigments production are on special substrates: Pseudomonas pyocyanin agar (PP), Pseudomonas agar fluprosceins (PF). Salts included in the substrates – magnesium chloride and potassium sulphate, stimulate the production of pigment. Pyocyanin secreted extracellularly stained the medium from light green to dark blue.

Production of chrome oxidase

In contrast to bacilli belonging to *Enterobacteriaceae,* species belonging to *Pseudomonaceae* produce cytochrome oxidase and decomposing glucose with oxygen. Cytochrome oxidase is the final enzyme of the respiratory chain in aerobic respiration. Cytochromes are found in aerobic, anaerobic and microaerophilic. Test for presence of cytochrome oxidase is important for the differentiation of many types of bacteria. It can be performed using two methods:

1) Reagents – 1 % solution of HCl di – or tetramethyl-p-phenylodiamin is dripped directly to colonies on Hugh-Leifson surface:
2) On a piece of paper bacterial mass is triturated collected from the substrate with glass rod, on bacteria 1% aqueous solution of HCl tetramethyl-p-phenylodiamin is dripped.

In both cases dark blue color (blue indolophenol is formed) of bacteria occurring within minutes indicates a positive reaction.

Saccharolytic properties

As with other bacteria biochemical identification, study of saccharolytic properties is carried out on a peptone medium supplemented with 1% of the corresponding substrate sugar (carbohydrate) and a pH indicator. A set of such several tests is called ordinary sugar series. During the metabolism of carbohydrates products are produced that acidify the medium and there is change of colour. During the biochemical identification of many bacteria, it is also important to determine ways of carbohydrate metabolism: oxidation or fermentation.

The test is performed on the buffered substrate-Leifson Hugh. In contrast to the above-described series of sugar, it contains five times less (only 0.2%) peptone. Addition to the medium 0.3% agar, gives it a semi-liquid consistency. The substrate contains 1 % carbohydrate and bromothymol blue, which at neutral pH is green.

The medium is poured out into the test tubes forming the high column. Prior to inoculation, in order to get rid of the residual oxygen, the medium was incubated at 100^0C 30 minutes. After cooling test strain is inoculated to two test tubes with the medium. After the culture, one of the test tubes is covered with a layer of liquid paraffin. Cultures are carried out at 35-37 ^{0}C for 2-5 days.

The test tube uncovered with paraffin below substrate there is a change of medium colour from green to yellow (aerobic conditions) and no change in the test tube covered with paraffin (anaerobic conditions).

Substrate colour change to yellow in both tubes indicates the distribution of carbohydrate fermentation. This process is independent of oxygen and occurs in both aerobic and anaerobic conditions.

Bacteria cultures of non-distributed carbohydrates do not change substrate colour. During the propagation of the test tribes of bacteria alkalization of cultures can become, resulting in formation of blue color of the substrate.

Table 3: The most important biochemical properties of *P. aeruginosa*.

Carried out feature	Result
Cytochrome oxidase	+
Oxidation of glucose	+
Oxidation of xylose	+
Oxidation of lactose	-
Growth at 42 ^{0}C	+
Growth at 4 ^{0}C	-
Hydrolysis of esculin	-
Denaza	-
Indole	-
Reduction of NO_3 to NO_2	+
Reduction of NO_3 to O_2	+
Decarbolsylation of lysine	-
Pigmemts production	
Pyocyanin – Z to N	+/-
Fluorescein – F	+
Cilia (movement)	+
Growth in McConkey's substrate	-/+

For diagnostic purposes, methods using molecular biology are developed. A very promising method seems to be the SS-PCR (species-specific), in which conservative, species-specific sequences for *P. aeruginosa* in the 16SrRNA [8] are specified.

The role of *P. aeruginosa* in environment

Bacteria of *Pseudomonas* group, due to low requirements, are met everywhere: in soil, water, wastewater and air. They represent about 90 % of waste water micro flora. This is because that tribes of this species are extremely resistant to disinfectants and antimicrobial agents. Natural habitat of these bacteria are therefore also the surface water of varying degrees of contamination. They are usually the first to colonize new places if there are mineral salts, organic acids or carbohydrates. They can often use a wide variety of organic substrates, non-degraded by other bacteria, including heterocyclic and aromatic compounds. The presence of *Pseudomonas* can be identified by the formation of water-soluble dyes, i.e. blue-green pyocyanin and yellow-green fluorescent pigments. Some of the secreted pigments act as siderophore [3]. Some species of *Pseudomonas* incompletely oxidize carbohydrates and release acid derivatives (gluconic acid, acid, 2-ketogluconic) into the environment.

A man is also a reservoir for *P. aeruginosa*. Carriers in the gastrointestinal tract occurs in `5 % of the population. In recent years, the presence of these bacilli in the feces of people and the environment has greatly increased, due to the increasingly widespread use of antibiotics in humans and animals.

Low nutrient requirements make it possible to proliferate in water poor in organic carbon. This means the risk of infection in food, pharmaceutical, cosmetic, and even in the hospital, even by distilled water as an ingredient in medicines, cosmetics, etc. [9]. For these reasons, apart from the group of bacteria *E. coli*, *Pseudomonas aeruginosa* bacilli, in addition to *Enterococcus faecalis* and *Clostridium perfringen*, are additional indicators of water pollution.

Participation of P. aeruginosa in bioremedition

Due to the high efficiency of microbial surfactants (biosurfactants), they are increasingly used to remove of heavy metals (lead, zinc, copper and cadmium) from soil, and then to create lasting connections with them. This type of action is performed, among others with the use of

biosurfactants of rhamonolipides obtained from cultures of *P. aeruginosa* [9].

Microbes also change iron in a soluble form. The resulting complexes containing Fe^{3+} are transported into the bacterial cell. Substances of this type are called siderophore. In terms of chemical structure siderophore are included in phenolic compounds and hydroksamats. They are, almost without exception, water-soluble substances, binding iron with a very high specificity and affinity. The first of these groups contains enterobactin having six phenolic hydroxyl groups. Some enteric bacteria emit this compound.

Greenish fluorescent pigment secreted by *P. aeruginosa* are of siderophore nature as well.[3].

P. aeruginosa is one of the microorganisms that produce useful egzopolisaharide. One of them is alginate (combination of 1,4 glucoside mannouronic acids) found in seaweed.

It is used to produce ice cream, instant puddings and custards. It has also application as a surface for paper and textiles production as well as a hydrophilic shield of plants roots and healing [3].

Biofilms

Phenomenon of high sanitary and economic importance is vegetation of microbial in the form of biofilms. In contrast to the growth of single cells called planktonic, biofilm is a complex of colony interconnected with network of canals and proliferating in the matrix surrounding space filled with fluid. Proteome of bacteria in the biofilm is different from planktonic bacteria. Moreover, the proteome is dependent on the nature of the substrate.

Currently there are 3 main types of biofilm structure:

1) Flat, virtually two-layer structure. Carefully examined on dental board appear to be qualitatively heterogeneous, because they are even created by over 500 species of bacteria belonging to more than 30 families;
2) Micro colonies forming storey structures. They are surrounded by the matrix structure of the compounds of the polymers. They look like a column surrounded by a liquid phase. Other organisms, such as protozoa, can occur in it. It is a model "heterogeneous mosaic", which is produced by pathogenic organisms as well. *P. aeruginosa* forms a type of biofilm in the lungs of people with cystic fibrosis;
3) Model of the fungus. Short stem supporting a much larger part of the top is created. Multiple channels combined by pores with the external environment run over the whole.

Biofilm formation resembles the formation of fruit bodies of mucous bacteria. Maturation and development depend on the availability of nutrients, osmolarity, oxygen concentration inside the biofilm, and reaction (pH).

As far as *P. aeruginosa* is concerned philus type IV (TFP) are involved in micro colonies creation. *P. aeruginosa* subpopulation producing TFP can even build colonies similar to the cap of the fungus.

In the development of single-species biofilms formed among others by *P. aeruginosa*, as well as in multi-species biofilms an interesting phenomenon of activation of cell death program that runs with the participation of intercellular signaled by specific regulatory factors were observed.

During maturation of the biofilm a single bacteria or large portions of the biofilm are continuously discharged. The biofilms formed by *P. aeruginosa*, cell loss occurs as a result of changes in micro colonies, after reaching the appropriate size (approximately 80 microns) or when cell density reaches a critical value. Bacteria showing moveable features wander inside the colony, leaving a wall built of non-moveable bacteria. This functional differentiation of cells (belonging to the same species) is reversible and is probably associated with their unequal access to oxygen and nutrients.

Biofilm is an interesting natural microbiology phenomenon. The knowledge gained from the culture of microorganisms propagated under optimal natural conditions, is different from the knowledge we can gain from the proliferation of microorganisms in the laboratory.

Biofilm formation in infected body significantly increases the resistance of *P. aeruginosa* to antibiotics and protects bacteria in biophilies against phagocytes. *P. aeruginosa* in the biofilm overcomes additionally the processes of phagocytosis

by proteins effector of the secretion system type III (Exos and ExoL) [6].

Pathogenicity of *P. aeruginosa*

The virulence factors

The effectiveness of pathogenic of any pathogen is determined by the possessed virulence factors. In the case of *P. aeruginosa* the most important virulence factors are:

1. Fimbriae (philus)
Constructed of thousands of protein subunits philin are important factors of virulence of many species of gram-negative bacteria. They participate in the processes of the impact of pathogen – host, pathogen – pathogen, and determine bacterial motility [4]. Their activity depends on many features of the bacterial cell. In addition to motility and adhesion facilitating, include the ability to create micro colonies and biofilm, invasiveness, and phages binding can be mentioned.

2. Envelope
It is made of alginate, and found only in pathogenic tribes. Envelope has a particular affinity with lung epithelial cells [3].

3. Toxin
P. aeruginos exotoxin works inside the targeted cells by inhibiting protein synthesis in them, which leads to the whole organs damage [5].

4. Alkaline proteases of P. aeruginosa hydrolyze elastin protein being in large amounts in the lungs and the walls of blood vessels. Another enzyme – thermolabile heat-phospholipase C (lecithinase) disrupts the phospholipids in the infected cells.

5. Enterotoxin is present in tribes of *P. aeruginosa* in tropical climates.

6. Thermostable glycolipid causing blood cells hemolysis.

7. Leucidin belongs to hemolisine and is an important virulence factor.

8. Mucous coating
On the surface of some tribes mucinous material indicating the protective properties of the bacteria is observed.

9. Egzopolisaharide
An important factor in the pathogenesis of infections are egzopolisaharide [4]. They participate in creating micro colonies *P. aeruginosa* in the lungs of patients with cystic fibrosis [5].

10. *P. aeruginosa* is able to resist phagocytosis by having proteins that protect it frominternalisation [6].

Forms of *P. aeruginosa* infections

With such a rich set of virulence factors *P. aeruginosa* produces many forms of infection.

Infections caused by *P. aeruginosa*, as well as by some protozoa, yeasts and other fungi are very rare in people with a properly functioning immune system [3]. Most are opportunistic infections.

Infectious of lung, eye, urinary tract, heart, central nervous system, other opportunistic and infections have been described in literature so far. [8].

P. aeruginosa is most frequently mentioned as the perpetrators of clinical hospital-acquired infections [5] and bacteremia in patients with severe burns [7].

1. Infections in lungs.
Pneumonia and various forms of infections of the upper and lower respiratory tract.

In particular, they are common in people with cystic fibrosis [3] and nosocomial pneumonia: after intubation, artificial ventilation, aspiration pneumonia and pneumonia in patients hospitalized in intensive care units.

2. Eye infections.
P. aeruginosa is described among others as the etiologic agent of infections of the cornea and conjunctiva leading to various forms of inflammation, and serious infections of eye-ball.

3. Infections of urinal system.
P. aeruginosa is the most common factors of inflammation of the prostate (prostate cancer), acute pyelonephritis and nosocomial urinary tract infections associated with urinary catheters.

4. Infections of heart muscle.
Infections usually occur after invasive diagnostic and therapeutic intervention.

5. Infections in central nervous system.
Infections in this area usually lead to inflammation of meningitis and even brain abscesses in older people.

6. Infections of skin and skin structure.
P. aeruginosa is essentially unable to cause infection of the skin healthy, but it can cause serious infections in people with compromised immune systems resulting from genetic defects, treatment or certain virus infection (HIV) [5]. In addition to these infections, *P. aeruginosa* is considered among others to be etiological factor for inflammation of hair follicles and sweat glands [8].

7. Wound infections.
They lead to suppurative inflammation of burn wounds (infections is often complicated by *S. sureus*), and after the cuts of contaminated soil. In hospitals there are often the surgical site infection and infections after organ transplantation

8. Due to the importance of clinical relevance a separate discussion of chronic *P. aeruginosa* infection in human patients with cystic fibrosis is required.

Cystic fibrosis (CF) is a monogenic autosomal disease associated with gene defects of the transmembrane transition regulator (CFTCR-cystic fibrosis transmembrane conductance regulator).

The main problem in this disease are chronic, unmanageable bacterial infections. The main culprit of these infections is *P. aeruginosa* [3].

CFTCR protein is a receptor that causes the recognition and absorbing of *P. aeruginosa* to epithelial cells. Absorbed bacteria cause inflammation, in which the body reacts by removing of epithelium infected by *P. aeruginosa*.

Tribes of *P. aeruginosa* isolated from patients have a tendency to produce exopolysaccharide alginate discussed above (acylated polymer of mannuronic acid and glucuronic). Alginate allows bacteria to avoid non-specific host defense mechanisms.

Tribes isolated from patients produce alginate, called mucoidal, very quickly return to non-mucoidal phenotype [7]

In addition, *P. aeruginosa* can cause various forms of other infections. These infections are reported in people suffering from leukemia. *P. aeruginosa* can cause septicemia (after the fracture of bones and joints), ear infections in swimmers and divers (the swimmer ear), foot infections in diabetic (so-called diabetic foot).

P. aeruginosa and antibiotics

In addition the minimum nutritional requirements P. aeruginosa is characterized by significant resistance to many antibiotics.

The origin of the phenomenon of antibiotic resistance of pathogens is to be found in soil, to be more precisely, in soil bacteria that encode genes for antibiotic production. Closely related transposons may be conveyors of resistivity genes. The plasmids transposons using *P. aeruginosa* can be built to bacteria that infect the human body [10].

Another mechanism of resistance is the impermeability of the cell membrane of bacteria to antibiotics. It is this natural immunity *P. aeruginosa*, as it is claimed, to be associated with poor penetration of antibiotic into the bacterial cell.

Very worrying phenomenon for antibiotic therapy perspective is increasingly frequent appearance of tribes in the hospital environment, among others. *P. aeruginosa* – producing beta – lactamase class B [10].

The sensitivity of *P. aeruginosa* to antibiotics

P. aeruginosa is sensitive to the following antibiotics:

1) Antibiotics penicillin derivatives. Semisynthetic penicillins;
2) Aminoglycosides;
3) Quinolones;
4) Cephalosporins;
5) Monobactam (aztreonam);
6) Carbapenems (imipenem) [2];
7) Polymyxin.

Antibiotics penicillin derivatives. Semisynthetic penicillin:

1) Carbenicillin, semi-synthetic penicillin;
2) Tycarcylin carboxy derivate penicillin (resistant tribes are present);
3) Azlocylin (ureidopenicylin) betalactam antibiotic with a broad spectrum of activity with particular activity against *P. aeruginosa*. It may be associated with an aminoglycoside.
4) Mezlocylin. Betalactam antibiotic of acyloureidopenicylin group with a broad spectrum of activity. It is active against *Pseudomonas* as well;
5) Piperacillin. Betalactam antibiotic. Piperazine derivative of penicillin. The broad spectrum of activity, including in relation to *P. aeruginosa*.

Aminoglycosides

Among aminoglycosides, in preventing from *Pseudomonas* infections, gentamicin has been used for the longest period of time due to a broad spectrum of action. In the case of *P. aeruginosa* it is often used it in combination with beta – lactam antibiotic [10].

Quinolones

Despite the effectiveness in combating *P. aeruginosa* infections, an increasing number of resistant tribes, even to the new quinolones: ciprofloxacin and ofloxacin. In addition *P. aeruginosa* shows a lack of sensitivity to nalidixic acid, a typical representative of the older quinolones. The mechanism of resistance accumulation is the mutational changes leading to the formation of a "pump" MDR (multi-drug resistance), effectively removing quinolones from bacterial cell [10].

The use of quinolones in *P. aeruginosa* infections monotherapy also leads to selection of tribes cross resistant to imipenem belonging to the carbapenem beta-lactam antibiotics, closely related to penicillin and cephalosporins. However, strains resistant to quinolones and imipenem are sensitive to penicillin and cephalosporins [2].

The first and second generation of cephalosporins are inactive against the bacteria of *Pseudomonas*. The third and fourth generation act on gram-negative bacilli, including ceftazidime and cefoperazone for *Pseudomonas* type, with four runs on tribes resistant to the third generation producing chromosomal cephalosporinases.

P. aeruginosa resistance to ceftazidime occurs only at sites where this antibiotic is widely used in departments such as treating patients with cystic fibrosis, hematology, burn and intensive care units.

Semisynthetic syderophoric cephalosporin have especially high activity against *P. aeruginosa* [10].

In some cases one observed a paradoxical effect of the low dose of antibiotics manifested by the increased ability to adhesion of *P. aeruginosa* to the surface of cells.

Monobactam (aztreonam)

These are beta-lactam antibiotics. Among them aztreonam is used in the treatment of bacterial infections. It presents bactericidal activity only against gram-negative aerobic bacteria such as *Pseudomonas*. It is highly resistant to betalactamase of gram-negative bacteria. It shows a cross allergic reaction.

Carbapenems (imipenem)

They are betalactam antibiotics, the affinity of penicillins and cephalosporins, but chemically different. Carbapenems are used in the empirical treatment of among others severe infections caused by *P. aeruginosa*, and may be safely associated with aminoglycosides [2].

Imipetem is such carbapenem that shows resistance to these betalactamase and thus it is found in medicine. In the renal tubular epithelium it is degraded by two pepdisae hydroxy. To avoid this degradation it is associated with it which cilastatin being competitive reversible dehyrogenase inhibitor. Cilastatin also protects kidneys from the toxic effects of the antibiotic. Side effects are similar to those described for penicillin and cephalosporins [8].

Polymyxin

Polymyxin are the most commonly used cyclic peptide antibiotics produced by *Bacillus polymyxa*. These antibiotics and colistin have a limited spectrum of activity including only some gram-negative bacilli, mainly *Pseudomonas spp* [10].

Bicyclomycine of inhigitors belongs to the cell wall synthesis by inhibiting synthesis of lipopropeid of gram-negative bacteria. *P. aeruginosa* is extremely sensitive to this antibiotic [10]. It is used to treat urinary tract infections [2].

Current interest in *P. aeruginosa* in cosmetical microbiology

Current trends in studies on *P. aeruginosa* in cosmetics concerns looking for new recipes, natural preservatives, permitting the growth of microorganisms and spoilage of cosmetics.

Among other things, an assessment of the effectiveness of the preservative composition of the composition of water, silicone, glycol, siloxane with 1% retinol palminynian.

It was shown that after 7 days of proliferation to the conservation composition, all test organisms, including *P. aeruginosa* were eliminated, with the exception of *Aspergillus niger*, which proved to be viable even after 28 days [11].

In search of preservatives one also assessed the effectiveness of antimicrobial colistin as an alternative preservative cosmetics. This natural sugar derived from plants, fruits and vegetables has antimicrobial properties. It was determined that the MIC colistin for microbial testing among others *P. aeruginosa* was 1 % to 1.25 % [12].

Similarly one assessed the antimicrobial activity of oils of lavender, tea tree and lemon in body and bath lotions. In relation to *P. aeruginosa*, these oils were effective at a concentration of 1% in liquids and 0.5% in lotions. In a mixture of synthetic preservative MDM hydantoin and 3-iodo-2-butyl carbamate efficiency were achieved already at a concentration of 0.1 % and 0.3 % [13].

In combination with phenylethanol the most popular preservative efficacy was achieved at low concentrations. Such preservation has many advantages. The effectiveness of antimicrobial preservatives, perfumes and known allergens of different power [diazolidinyl urea, Methylchloroisothiazolinone / Methylisothiazolinone (MCI / MI), Methylisothiazolinone (MI) and Phenoxyethanol] examined separately or in various associations of two or three preservatives together.

Preservatives were tested to determine the MIC and the possible synergy using a fractionation inhibitory concentration. MCI / MI was the only preservative showing low levels of MIC against all four microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*. Various associations have the additional impact on preservatives on microorganisms. No association of preservatives has no effect on each of them. Challenge tests with different concentrations and associations have been carried out on cosmetic creams. Diazolidinyl urea and MCI / MI alone proved ineffective against *C. albicans* in the challenge test at concentrations of more than 16 times higher than the observed value of the MIC. When phenoxyethanol associated with other allergenic preservatives diazolidinyl urea, MCI / MI or MI, cosmetic cream was adequately maintained at concentrations below the preserving MIC, and 10-20 times less than the maximum permissible concentration. Using the combination of preservatives, effective preservance can be achieved at lower concentrations of allergenic preservatives [14,15].

Considering that even minimal contamination of pathogenic bacteria are undesirable, methods of molecular biology were applied. One tested successfully ability to detect contamination of calcium carbonate powder used in cosmetics with bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa, Salmonella spp and Escherichia coli*. It was estimated that the analyst can be very useful for the detection of a variety of contaminants in both raw materials and finished cosmetic products. Molecular analyst can be valuable in detecting the presence of undesirable microorganisms in cosmetic products with enhanced microbiological purity requirements (cosmetics for children and eyes). Guidelines for the quantitative determination of bacterial contamination were developed [14].

Conclusion

The presented characteristics of bacteria *P. aeruginosa* shows the scale of the risks for the use of cosmetics poorly preserved and could be a medium for its growth. Emergence of these threats is supported by such properties of this bacterium as: the minimum nutritional requirements, very rich and diverse set of virulence factors which demonstrate their abilities in the form of pathogenic infections or complications of diseases of many organs and systems of the human body. In these respects *P. aeruginosa* shows a typical opportunistic bacterium and is superior

to other gram-negative indicator knobs, like the very important sanitary E. coli and bacilli of Salmonella gene.

Current trends in studies of *P. aeruginosa* in cosmetics reflect the need to explore the composition of the required preservative efficacy with minimal concentrations.

The future belongs to genetic testing and methods for detecting small quantities of impurities of cosmetics with the required high microbiological safety.

References:

1. Amaral L.F.B., Camilo N.S., Pereda M.D.C.V., Levy C.E., Moriel P., Mazzola P.G.: Evaluation of antimicrobial effectiveness of C-8 xylitol monoester as an alternative preservative for cosmetic products. International Journal of Cosmetic Science. 2011; 33 (5): 391-397.
2. Baj J., Markiewicz Z.: Molecular Microbiology of bacterial. Polish Scientific Publishers PWN, 2006
3. Błaszczyk M.K.: Microorganisms in Environmental Protection. Polish Scientific Publishers PWN. 2007
4. Chorilli M., Leonardi G.R., Salgado H.R.N., Scarpa M.V.: Evaluation of preservative effectiveness of liquid crystalline systems with retynil palmitate by the challenge test and D-value. Journal of AOAC International. 2011; 94 (1): 118-127
5. Dzierżanowska D: Practical antybiotics therapy. Alf-media press, Bielsko Biała, 1994
6. Di Maiuta N., Schwarzentruber P.: Molecular detection of bacteria in calcium carbonate powder used in cosmetic formulations. International Journal of Cosmetic Science. 2011; 33 (5): 426-431
7. Kunicka-Styczynska A., Sikora M., Kalemba D.: Lavender, tea tree and lemon oils as antimicrobials in washing liquids and soft body balms. International Journal of Cosmetic Science. 2011; 33 (1): 53-61
8. Libudzisz Z., Kowal K., Żakowska Z.: Technical Microbiology. Polish Scientific Publishers PWN. Warszawa 2009
9. Lundov M.D., Johansen J.D., Zachariae C., Moesby L.: Low-level efficacy of cosmetic preservatives. International Journal of Cosmetic Science. 2011; 33 (2): 190-196
10. Markiewicz Z., Kwiakowski Z.K.: Bacteria, antibiotics and medicines resistance. Polish Scientific Publishers PWN 2006
11. Nicklin J., Graeme-Cook K., Killington R.: Microbiology. Polish Scientific Publishers PWN, Warszawa,2004
12. Rozporządzenie Ministra Zdrowia z dnia z dnia 23 grudnia 2002 roku w sprawie określenia procedur pobierania próbek kosmetyków oraz procedur przeprowadzenia badań laboratoryjnych.
13. Szewczyk E.: Bacteriological Diagnosis. Polish Scientific Publishers PWN, Warszawa, 2005
14. Schleger H.G.: General Microbiology. Polish Scientific Publishers PWN, Warszawa, 2003
15. Zaremba L.: Medical Microbiology. Wydawnictwo Lekarskie PZWL. Warszawa.1997.