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Biogenic polyamines and genital gonococcal infection: facts and hypotheses

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ABSTRACT

Genital gonorrhea is one of the most common sexually transmitted diseases with significant gender differences in its clinical course. Laboratory verification of the diagnosis is associated with great difficulties in the cultivation and identification of the pathogen. Moreover, the diagnosis of female gonorrhea is a serious problem due to mild symptoms of the disease. Currently, a promising trend in the diagnosis of inflammatory diseases of reproductive organs is biochemical analysis of vaginal and sperm fluids, which have a rich component composition. Biogenic polyamines can be synthesized by both pro- and eukaryotic cells. These polycations are present in semen and vaginal fluid and can have a significant effect on various cell structures and functions. In this regard, the qualitative and quantitative composition, the level and ratio of these components and their changes can have a diagnostic value for infections of the genital tract.

The aim of the review was to analyze current information on the role of biogenic polyamines in the physiological and biochemical potential of *Neisseria gonorrhoeae* and their participation in the development of genital gonococcal infection, taking into account the influence of sexual differences and a number of related factors. Special attention was paid to the origin and possible functional role of polyamines in the genital tract of men and women. As a result, taking into account the spectrum, origin and ratio of polyamines in the corresponding fluids, we formulated a hypothesis: the manifestation of the process in case of infection in men is largely determined by the reactivity of eukaryotic cells, but not the metabolic activity of the microbiota of the reproductive tract. At the same time, the development of "female" gonorrhea is primarily determined by the state of the microbiocenosis of the cervical vaginal biotope.

Key words: genital gonococcal infection, *Neisseria gonorrhoeae*, biogenic polyamines, microbiota, biofilms, antibiotic resistance.

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Биогенные полиамины при генитальной гонококковой инфекции: факты и гипотезы

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РЕЗЮМЕ

Генитальная гонорея является одним из наиболее распространенных венерических заболеваний и характеризуется существенными гендерными различиями в его клиническом течении. Лабораторное подтверждение диагноза сопряжено с большими сложностями культивирования и идентификации возбудителя, а диагностика «женской» гонореи представляет серьезную проблему еще и в связи со стертой симптоматикой инфекционного процесса. На современном этапе перспективным направлением для диагностики воспалительных заболеваний репродуктивных органов признается изучение биохимического состава влагалищной и спермальной жидкостей, имеющих богатейший компонентный состав. Биогенные полиамины, которые могут синтезироваться как про-, так и эукариотическими клетками и в значительных количествах обнаруживаться в этих секретах, являются низкомолекулярными соединениями, оказывающими разнообразные эффекты на жизненно важные структуры и функции клеток обоих типов. В этой связи качественный и количественный состав, уровень и соотношение этих компонентов в секретах, с учетом изменения соответствующих показателей в динамике, могут иметь диагностический смысл при инфекционной патологии генитального тракта. Целью обзора явилось рассмотрение накопленной к настоящему времени информации о возможной роли биогенных полиаминов в физиолого-биохимическом потенциале *Neisseria gonorrhoeae* и их участии в развитии генитальной гонококковой инфекции с учетом влияния половых различий и ряда сопутствующих факторов. Особое внимание уделено происхождению и возможной функциональной роли полиаминов в генитальном тракте мужчин и женщин. В результате, с учетом спектра, происхождения и соотношения полиаминов, доминирующих в составе соответствующих секретов, сформулирована гипотеза о том, что манифестация процесса в случае инфицирования мужчин в большей степени обусловлена реактивностью эукариотических клеток, но не метаболической активностью микробиоты их репродуктивного тракта. В то время как развитие «женской» гонореи в первую очередь определяет состояние микробиоценоза цервикально-вагинального биотопа.

Ключевые слова: генитальная гонококковая инфекция, *Neisseria gonorrhoeae*, биогенные полиамины, микробиота, биопленки, антибиотикорезистентность.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с проведенным исследованием и публикацией настоящей статьи.

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INTRODUCTION

Gonococcal infection (GI) and its most common form, acute genital gonorrhea, is an infectious disease transmitted *primarily by sexual contact* and caused by *Neisseria gonorrhoeae* [1, 2]. Laboratory verification of the diagnosis is associated with difficulties in the cultivation and identification of the pathogen. Moreover, the diagnosis of “female” gonorrhea is a serious problem due to mild symptoms of the infectious process. Many researchers note that the promising trend in the diagnosis of inflammatory diseases of reproductive organs is the study of seminal and vaginal fluids [3–6], since they have a complicated biochemical composition comparable to blood. Even if we take into account only proteins with a molecular mass of 10–100 kDa, more than 200 of them were known in the sperm plasma of healthy fertile men in 1981 [7]. However, this huge diagnostic potential is used only to a small extent due to the lack of data on the physiological role of various components of these fluids in inflammation of the reproductive organs [4, 8–10]. Recently, a growing interest has appeared in biogenic polyamines (BPA) and their level, since these compounds are present in all pro- and eukaryotic cells and perform various functions, including protective ones. The content and balance of BPA change dynamically, which may be useful for diagnosis. The information about the involvement of BPA in the persistence of *N. gonorrhoeae* and development of genital GI is presented in a few studies and is often contradictory.

The aim of this article was to review the current information on the role of BPA in the physiological and biochemical potential of *N. gonorrhoeae* and their possible participation in the development of genital gonococcal infection, including the influence of gender differences and a number of related factors.

CHEMICAL STRUCTURE, SYNTHESIS AND TRANSPORT OF BPA

Biogenic polyamines are aliphatic hydrocarbons possessing two or more amino or imino groups and differing in the length of the carbon chain. The most widespread polyamines in living organisms are diamines: putrescine, cadaverine; triamines: spermidine, norspermidine; and tetraamines: agmatine and spermine [11].

Eukaryotic cells contain mainly spermidine and spermine, whereas putrescine is found in trace amounts. These polyamines are widely represented in various organs, tissues, and biological fluids of the human body both as free forms and as complexes with proteins and nucleic acids. The highest concentrations (millimoles) of spermidine and spermine are found in the sperm plasma of men [12], while in the vaginal discharge of women polyamines are detected in micromolar amounts [13]. On the contrary, the concentrations of putrescine and cadaverine in prokaryotic cells is the highest, spermidine is present in small amounts and spermine is practically not detected [11, 14, 15]. Some bacteria are capable of producing diaminopropane, homospermidine or norspermidine [11, 16]. The spectrum and quantity of BPA are significantly different in various species of microorganisms. Based on this fact, some authors have attempted to use polyamines as chemotaxonomic markers [16–18]. However, this approach does not seem to be promising, since the polyamine pool in the microbial cells depends on the age of the culture and growth conditions, such as composition and pH of the medium, temperature, aeration, etc. [19–21].

The intracellular content of polyamines is determined by several parameters: biosynthesis level, activity of degradation, as well as the intensity of the exchange of these components between the cell and the environment. Genes involved in the polyamine biosynthesis are found in genomes of many microorganisms; however, the active *de novo* synthesis of these compounds is mostly typical of gram-negative bacteria [22–24]. Putrescine can be synthesized in several ways: directly from ornithine by ornithine decarboxylase or from arginine by arginine decarboxylase with the formation of agmatine, or via N-carbamoyl putrescine [25, 26]. Spermidine is synthesized on the basis of putrescine and S-adenosylmethionine using the enzymes S-adenosylmethionine decarboxylase, aminopropyl transferase and spermidine synthetase [27, 28], or from putrescine and aspartate semialdehyde with involvement of carboxyspermidine decarboxylase and carboxyspermidine carboxylase [29–31]. Cadaverine synthesis in bacteria is realized by direct decarboxylation of lysine with lysine decarboxylase [32]. Among all the known polyamine synthesis enzymes, only arginine decarboxylase, that catalyzes the synthesis of agmatine (precursor of putrescine) from arginine, was detected in *N. gonorrhoeae* [33]. There are no data on the activity of this enzyme in *N. gonorrhoeae* in the available literature, which is probably determined by the difficulties in cultivating this microorganism under laboratory conditions and obtaining a sufficient amount of biomass.

The absence of other polyamine synthesis enzymes in gonococci can be partly explained by their specific habitats. In the urogenital tract of men, polyamines are present in large amount [12, 34], and bacteria can receive these compounds via transport. Many gram-negative bacteria are capable of transporting polyamines from the environment. For example, four BPA transport systems are found in *Escherichia coli* cells. Two of them (spermidine-preferential system PotDABC and putrescine-specific system PotFGHI) are ABC (ATP binding cassette) transporters [35]. Each of them consists of a periplasmic substrate-binding protein (PotD and PotF), two channel-forming transmembrane proteins (PotBC and PotHI) and membrane-associated ATPase (PotA and PotG) [36, 37]. The third transport system (PotE) catalyzes the uptake and excretion of putrescine [38]. Cadaverine is transported via the lysine-cadaverine antiporter CadB [39]. In contrast to enterobacteria, the transport of polyamines in gonococci is not sufficiently studied. The PotFGHI transport system was found in *N. gonorrhoeae*. It is similar to *E. coli* transport system and selectively transports spermine and spermidine, but not putrescine and cadaverine into the cell from the medium [40]. There is evidence that the agmatine/arginine antiport is encoded in the gonococcal genome. This system provides the uptake of agmatine in exchange for arginine, which is important for survival of *N. gonorrhoeae* in the acidic environment [33].

PARTICIPATION OF POLYAMINES IN PHYSIOLOGICAL PROCESSES

The fact that intracellular pool of BPA in many microorganisms reaches quite high (millimolar) concentrations even with growth on minimal media indicates the importance of these compounds for the cell physiology. Participation of polyamines in various cell processes is a consequence of their chemical structure. Under physiological conditions, amino groups in polyamine molecules are protonated and, thus, positively charged. Thereby, BPA are able to interact with negatively charged cell components, such as DNA, RNA, proteins [41, 42], membrane phospholipids, and cell wall structures [43]. Polyamines can modulate conformation and maintain structural and functional stability of the cell components, also indirectly through transport processes. It is shown that the concentration of BPA in the cell envelope changes in response to external influences that affect the porin activity and thus regulate the permeability of the outer membrane [44–46]. BPA are known to be involved in regulating nucleic acid synthesis, in particular, the replication process [27], as well as in maintaining the conformation of DNA and RNA [47, 48]. Of great importance is their ability to affect gene expression at the stages of transcription and translation [37, 49]. A group of genes which expression is regulated by polyamines at a translation level is called a “polyamine modulon” [50]. There is evidence that polyamines can affect the phosphorylation of specific proteins as well as modulate their degradation [49, 51].

The fact that polyamines are necessary for growth of *Neisseria* was first mentioned in 1952 [52]. It was later shown that polyamines along with other cations stabilized *N. gonorrhoeae* cells, preventing lysis. The same authors suggested the participation of BPA in the cell division process [53].

PARTICIPATION OF BPA IN ADHESION, BIOFILM FORMATION AND AGGREGATION OF BACTERIA

A lot of studies in recent years have indicated that BPA can be involved in the regulation of microbial adhesion, biofilm formation and aggregation [29, 54]. There is growing evidence that polyamines are able to influence biofilm formation by various commensal and pathogenic bacteria, including *E. coli, Bacillus subtilis, Staphylococcus aureus, Yersinia pestis, Enterococcus faecalis, Vibrio cholerae*, etc. [42, 55–57]. It is shown that not only polyamines of a host organism can regulate this process, but also the bacterial BPA. In particular, norspermidine, synthesized exclusively by bacteria, can inhibit the biofilm formation. However, the effect of BPA depends on the type of bacteria. For example, norspermidine effectively blocks the formation of a biofilm of *B. subtilis* and other bacterial species in laboratory conditions, and can cause its dispersion due to disruption of the matrix structure [58], but does not inhibit the formation of gonococcal biofilms and does not cause their dispersion [60].

N. gonorrhoeae cells in sperm, containing high concentrations of polyamines, must be adapted to such a medium. It is experimentally shown that seminal plasma and spermine inhibit adhesion of gonococci, but promote the aggregation of bacteria and formation of microcolonies [59]. A number of researchers indicate that spermine promotes the biofilm formation [60, 61]. These seemingly contradictory effects may be significant for successful colonization of biotopes and distribution of *N. gonorrhoeae*. Disruption of the contact of fixed cells with the surface can be important in the transmission of infection, in particular, when bacteria colonizing the male urethra leave it under the influence of sperm, providing their transition to the sexual partner [59].

However, it is possible that such results are determined by the specific methodological approaches to study the effect of seminal plasma. Biofilm formation is usually studied in a static system on a polystyrene surface by the method proposed at the end of the last century [62], while the adhesion of *N. gonorrhoeae* is studied using glasses or glass flow chambers [61]. On the other hand, it was shown that the specific twitching of *N. gonorrhoeae*, which is facilitated in the presence of seminal fluid, depends on the fluctuation of PilT pili. Despite the fact that pili are necessary for adhesion, the interruption of this process in the presence of spermine was not dependent on the presence of pili. Aggregation of gonococci in seminal plasma was also stimulated independently of these surface structures, which was demonstrated using mutants without pili [61]. It is assumed that BPA can greatly affect the ability of gonococci to form biofilms not only in the male, but also in the female urogenital tract due to a significant increase in spermidine and spermine concentrations after copulation with up to 15 mM spermine. [63].

It is believed that polyamines contribute to the transition of bacteria to the biofilm formation due to their toxic effects. This opinion is supported by the fact that spermine has the highest effect on this process among the four biogenic polyamines (putrescine, cadaverine, spermidine and spermine); at the same time it is the most toxic in high concentrations, especially in relation to gram-positive microorganisms [64, 65] . Meanwhile, it was experimentally shown that polyamines did not affect the viability of planktonic gonococci in physiological concentrations. The mutants with a damaged spermine and spermidine transport system (potHI) demonstrated that the transport of polyamines did not affect the biofilm formation. The authors of this study believe that, most likely, the key point is the effect of BPA on surface structures and bacterial aggregation of *N. gonorrhoeae* [40].

A study of the three-dimensional structure of *N. gonorrhoeae* biofilms showed that atypical films formed in the presence of spermine. They contained fewer cells and had a more condensed matrix, in which the channels providing the influx of nutrients and oxygen as well as excretion of metabolic products of bacteria were hardly visualized [40, 66]. Such biofilms, on the one hand, may be less viable; however, on the other hand, slow metabolism due to deficiency of nutrients can contribute to the development of persistent forms. At the same time, a study of the polyamine influence on mature biofilms showed that none of these compounds had an effect on their dispersion [60]. It should be noted that laboratory methods for assessing the ability of microorganisms to form biofilms, especially in the case of hardly cultivated bacteria, often do not take into account various aspects of the influence of environmental conditions that exist *in vivo* (pH, the presence of various substances in the medium, etc.). It can also be the cause of contradictory research results.

PROTECTIVE ROLE OF BPA IN THE GENITAL SECRETIONS

It is known that bacteria are able to synthesize BPA constitutively under normal conditions; however, many microorganisms activate the polyamine synthesis in response to environmental influences [21, 67, 68]. These studies were published at the end of the past century and focused on the fact that BPA perform protective functions. Polyamines are involved in adaptation to various types of starvation, heat and osmotic shock, oxidative stress, pH-shifts, and other stresses [20, 69, 70]. One of the most common types of negative effects for *N. gonorrhoeae* is an acidic environment. Lactic acid in the vaginal fluids of women protects against pathogenic microorganisms [71, 72]. The role of polyamines in the adaptation of *N. gonorrhoeae* to low pH values was first demonstrated as far back as 1976 [73]. Recent studies have shown that putrescine and cadaverine increased the survival of gonococci in the presence of lactic acid, stabilizing the cell wall and membrane [33]. These diamines are mainly produced by microorganisms and are actively formed in females with vaginosis [74]. Under these conditions, the chance of developing GI can increase greatly.

However, not only polyamines, but also their precursors are able to protect bacteria from the effect of acid. It was experimentally shown that arginine, glutamate, and lysine increase the resistance of *E. coli* and other bacteria to the lethal effect of low pH [75]. Only arginine and agmatine, but not glutamate or lysine, increase the acid resistance of gonococci [33]. The difference in the effects of amino acids may be explained by the fact that only one polyamine synthesis enzyme – arginine decarboxylase – is present in *N. gonorrhoeae* cells, which activity with the formation of agmatine is accompanied by a decrease in the medium acidity. Agmatine-arginine antiport promotes the exit of agmatine into the medium in exchange for arginine, providing resistance to the action of acid [71]. An increase in acid resistance under the arginine action is of practical importance, since a large amount of this amino acid is contained in seminal fluid (7.3 ± 1.5 mM) [76]. It is believed that after sexual intercourse, when genital secretions are mixed, the concentration of seminal arginine decreases slightly, depending on the volumes of ejaculate [77] and vaginal discharge [78]. Thus, it is possible that the amount of arginine in the seminal fluid is sufficient to provide the survival of gonococci and the colonization of the female vaginal–cervical biotope [79].

It is generally acknowledged that the composition of the vaginal microbiota is extremely diverse and can vary from a moderate microbial spectrum, characterized by predominance of a small number of lactobacillus species, to complex anaerobic communities that cause the development of bacterial vaginosis (BV) [80–83]. BV can have many negative effects on reproductive health, including an increased risk of sexually transmitted infections [84–86], HIV [87, 88], premature delivery [89], pelvic inflammation [90], and cervicitis [91]. With bacterial vaginosis, the content of putrescine and cadaverine in the vaginal secretions significantly increases, while the concentration of their precursors, amino acids, especially arginine and ornithine, decreases. Putrescine and cadaverine in this biotope are produced mainly by bacteria [74, 92, 93], as evidenced by the inhibition of their accumulation in the presence of metronidazole [94], which has a bactericidal effect.

It is interesting to note that various inflammatory diseases of male genital tract are accompanied by a decrease in the polyamine concentration in the ejaculate and a significant change in their ratio [95]. It is worth noting that even with pronounced bacteriospermia, the content of "bacterial" polyamines does not reach the values comparable to those in the vaginal fluid. Probably, the reason for this is the effect of acidity on the metabolism of polyamines, since the inducible BPA synthesizing enzymes have low optimal pH values [19], which are typical of the vagina. Polyamines in this case can be by-products that are formed due to bacterial protection against acid stress [96, 97]. On the other hand, it was shown that volatility of short chain polyamines increases with rising pH [98, 99]. It can also be the cause of low detectable concentrations of free putrescine and cadaverine in sperm plasma.

ALTERATION IN THE ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA IN THE PRESENCE OF BPA

There is evidence that polyamines contribute to the survival of microorganisms under antibiotic treatment. This is mainly shown on gram-negative bacteria, such as *E. coli, Salmonella enterica, Pseudomonas aeruginosa,* and others [69, 70, 100]. A decrease in their susceptibility to various groups of antibacterial agents (beta-lactams, aminoglycosides, fluoroquinolones) in the presence of BPA has been experimentally proven [69, 70, 101]. Since the fluids of the urogenital tract, especially in men, are rich in polyamines (spermine and spermidine), it is believed that these compounds could protect gonococci from antimicrobial agents. In the available literature, we did not find such data. Polyamines are shown to protect gonococci against cationic antimicrobial peptides (polymyxin B and LL-37), but there is no protective effect under ciprofloxacin, spectinomycin, and penicillin treatment [102]. The absence of the effect of polyamines on the *N. gonorrhoeae* susceptibility to antibiotics looks rather unexpected, since their protective action is associated with such universal mechanisms as deactivation of ROS, which are forming in the cells under antibiotic effect [70], reduction of antimicrobial transport due to a decrease of the porin channel permeability [45] and other mechanisms. No doubt, this issue requires further studying.

CONCLUSION

It is well known that genital GI proceeds differently in men and women. The "male" gonorrhea is characterized by manifested symptoms and pus-like discharge. On the contrary, in women, the disease, as a rule, is not accompanied by typical symptoms, and they often learn about GI after infecting their sexual partner. In this respect, the differences in the spectrum and content of BPA in the ejaculate and vaginal discharge seem to be interesting. It is supposed that the manifestation of the process in the first case is largely determined by the reactivity of eukaryotic cells, and not by the metabolic activity of the microbiota of the male reproductive system. At the same time, the course of "female" gonorrhea is primarily determined by the state of the microbiota of the cervical–vaginal biotope, which is indirectly marked by the origin of the dominating polyamines. We believe that the diagnostic, prognostic and differentiating value of the content and spectrum of BPA in the reproductive tract of men and women with genital gonococcal infection and other sexually transmitted infections requires clarification.

REFERENCES

1. Stevens J.S., Criss A.K. Pathogenesis of *Neisseria gonorrhoeae* in the female reproductive tract: neutrophilic host response, sustained infection, and clinical sequelae. *Curr. Opin. Hematol*. 2018; 25 (1): 13–21. DOI: 10.1097/MOH.0000000000000394.
2. Hook E., Handsfield H. Gonococcal infections in adults. In: Holmes K.K., Sparling P.F., Mardh P.-A., Lemon S.M., Stamm W.E., Piot P., Wasserheit J. N. (ed.). Sexually transmitted diseases 3rd ed. New York: McGraw-Hill, 1999: 451–466.
3. Lipatova N.A. Laboratory criteria for ejaculate fertility. *Russian Clinical Laboratory Diagnostics.* 1998; (5): 11–15 (in Russ.).
4. Evdokimov V.V., Erasova V.I., Orlova E.V. Protein factors of fertility. *Andrology and Genital Surgery*. 2004; (4): 30–32 (in Russ.).
5. Boe-Hansen G.B., Fedder J., Ersboll A.K., Christensen P. The sperm chromatin structure assay as a diagnostic tool in the human fertility clinic. *Hum. Reprod*. 2006; 21 (6): 1576–82. DOI: 10.1093/humrep/del019.
6. Krawetz S.A., Kruger A., Lalancette C., Tagett R., Anton E., Draghici S., Diamong M.P. A survey of small RNAs in human sperm. *Hum. Reprod*. 2011; 26 (12): 3401–3412. DOI: 10.1093/humrep/der329.
7. Edwards I.S. Postvasectomy testing: reducing the delay. *Med. J. Aust*. 1981; 1 (12): 649.
8. Boyko O.V., Terentyev A.A., Nikolaev A.A. Methodological aspects of the use of hydrochloric acid spermine and spermidine to identify uropathogenic microflora. *Russian Journal of Human Reproduction*. 2010; (3): 77–79 (in Russ.).
9. Al-Shukri S.Kh., Bobkov Yu.A., Galkina O.V., Gorbachev A.G., Kozlov V.V., Tomolyan A.A. Informative immunological analysis of blood and ejaculate in the diagnosis of chronic prostatitis. *Urology*. 2002; (2): 24–27 (in Russ.).
10. Pattinson R.C., Snyman L.C., Macdonald A.P. Evaluation of a strict protocol approach in managing women with severe disease due to abortion. *S. Afr. Med. J.* 2006; 96 (11): 1191–1194.
11. Michael A.J. Polyamines in eukaryotes, bacteria, and archaea. *J. Biol. Chem*. 2016; 291 (29): 14896–14903. DOI: 10.1074/jbc.R116.734780.
12. Lefèvre P.L., Palin M.F., Murphy B.D. Polyamines on the reproductive landscape. *Endocr. Rev*. 2011; 32 (5): 694–712. DOI: 10.1210/er.2011-0012.
13. Mendonca K., Costa C., Ricci V., Pozzi G. Enzymatic assay to test diamines produced by vaginal bacteria. *New Microbiol*. 2015; 38 (2): 267–70.

14. Herbst E.J. Weaver R.H., Keister D.L., The gram reaction and cell composition: diamines and polyamines. *Arch. Biochem. Biophys*. 1958; 75 (1):171–7.
15. Pegg A.E., Michael A.J. Spermine synthase. *Cell Mol. Life Sci*. 2010; 67 (1): 113–21. DOI: 10.1007/s00018-009-0165-5.
16. Hamana K., Matsuzaki S. Polyamines as a chemotaxonomic marker in bacterial systematics. *Crit. Rev. Microbiol*. 1992; 18 (4): 261–283. DOI: 10.3109/10408419209113518.
17. Hamana K., Nakagawa Y. Polyamine distribution profiles in newly validated genera and species within the *Flavobacterium-Flexibacter-Cytophaga-Sphingobacterium* complex. *Microbios*. 2001; 106 (2): 105–116.
18. Hosoya R., Hamana K., Niitsu M., Itoh T. Polyamine analysis for chemotaxonomy of thermophilic eubacteria: polyamine distribution profiles within the orders *Aquificales, Thermotogales, Thermodesulfobacteriales, Thermales, Thermoanaerobacteriales, Clostridiales and Bacillales*. *J. Gen. Appl. Microbiol*. 2004; 50 (5): 271–287.
19. Tabor C.W., Tabor H. Polyamines in microorganisms. *Microbiol. Rev*. 1985; 49 (1): 81–99.
20. Tkachenko A.G., Pshenichnov M.R., Salakhetdinova O.Ya., Nesterova L.Yu. Role of putrescine and potassium transport in regulating the topological state of DNA during adaptation of *Escherichia coli* to temperature stress. *Microbiology*. 1998; 67 (5): 601–606 (in Russ.).
21. Tkachenko A.G., Shumkov M.S., Akhova A.V. Adaptive functions of polyamines in response to sublethal concentrations of antibiotics. *Microbiology*. 2009; 78 (1): 32–41 (in Russ.).
22. Morris D.R., Koffron K.L. Putrescine biosynthesis in *Escherichia coli* regulation through pathway selection. *J. Biol. Chem*. 1969; 244 (22): 1094–1099.
23. Glansdorff N. Biosynthesis of arginine and polyamines. *EcoSal Plus*. 2004; 1 (1). DOI: 10.1128/ecosalplus.3.6.1.10.
24. Michael A.J. Biosynthesis of polyamines and polyamine-containing molecules. *Biochem. J*. 2016; 473 (15): 2315–2329. DOI: 10.1042/BCJ20160185.
25. Pegg A.E. 2006 Regulation of ornithine decarboxylase. *J. Biol. Chem*. 2006; 281 (21): 14529–14532. DOI: 10.1074/jbc.R500031200.
26. Burrell M., Hanfrey C.C., Murray E.J., Stanley-Wall N.R., Michael A.J. Evolution and multiplicity of arginine decarboxylases in polyamine biosynthesis and essential role in *Bacillus subtilis* biofilm formation. *J. Biol. Chem*. 2010; 285 (50): 39224–39238. DOI: 10.1074/jbc.M110.163154.
27. Tabor C.W., Tabor H. Polyamines. *Annu. Rev. Biochem*. 1984; 53: 749–90.
28. Pegg A.E. S-Adenosylmethionine decarboxylase. *Essays Biochem*. 2009; 46: 25–45. DOI: 10.1042/bse0460003.
29. Lee J., Sperandio V., Frantz D.E., Longgood J., Camilli A., Phillips M.A., Michael A.J. An alternative polyamine biosynthetic pathway is widespread in bacteria and essential for biofilm formation in *Vibrio cholerae*. *J. Biol. Chem*. 2009; 284 (15): 9899–9907. DOI: 10.1074/jbc.M900110200.
30. Hanfrey C.C., Pearson B.M., Hazeldine S., Lee J., Gaskin D.J., Woster P.M., Phillips M.A., Michael A.J. Alternative spermidine biosynthetic route is critical for growth of *Campylobacter jejuni* and is the dominant polyamine pathway in human gut microbiota. *J. Biol. Chem*. 2011; 286 (50): 43301–43312. DOI: 10.1074/jbc.M111.307835.
31. Ohnuma M., Terui Y., Tamakoshi M., Mitome H., Niitsu M., Samejima K., Kawashima E., Oshima T. N1-aminopropylagmatine, a new polyamine produced as a key intermediate in polyamine biosynthesis of an extreme thermophile *Thermus thermophilus*. *J. Biol. Chem*. 2005; 280 (34): 30073–30082. DOI: 10.1074/jbc.M413332200.
32. Nagano T., Kikuchi Y., Kamio Y. High expression of the second lysine decarboxylase gene, *ldc*, in *Escherichia coli* WC196 due to the recognition of the stop codon (TAG), at a position which corresponds to the 33th amino acid residue of sigma38, as a serine residue by the amber suppressor, *sup*D. *Biosci. Biotechnol. Biochem*. 2000; 64 (9): 2012–2017. DOI: 10.1271/bbb.64.2012.
33. Gong Z., Tang M.M., Wu X., Phillips N., Galkowski D., Jarvis G.A., Fan H. Arginine- and Polyamine-Induced Lactic Acid Resistance in *Neisseria gonorrhoeae*. *PLoS One*. 2016; 11 (1): e0147637. DOI: 10.1371/journal.pone.0147637.
34. Coffino P. Polyamines in spermiogenesis: not now, darling. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97 (9): 4421–4423.
35. Pistocchi R., Kashiwagi K., Miyamoto S., Nukui E., Sadakata Y., Kobayashi H., Igarashi K. Characteristics of the operon for a putrescine transport system that maps at 19 minutes on the *Escherichia coli* chromosome. *J. Biol. Chem*. 1993; 268 (1): 146–152.
36. Igarashi K., Ito K., Kashiwagi K. Polyamine uptake systems in *Escherichia coli*. *Res. Microbiol*. 2001; 152 (3-4): 271–278.
37. Igarashi K., Kashiwagi K. Characteristics of cellular polyamine transport in prokaryotes and eukaryotes. *Plant Physiol. Biochem*. 2010; 48 (7): 506–512. DOI: 10.1016/j.plaphy.2010.01.017.
38. Kashiwagi K., Shibuya S., Tomitori H., Kuraishi A., Igarashi K. Excretion and uptake of putrescine by the PotE protein in *Escherichia coli*. *J. Biol. Chem*. 1997; 272 (10): 6318–6323.
39. Soksawatmaekhin W., Kuraishi A., Sakata K., Kashiwagi K., Igarashi K. Excretion and uptake of cadaverine by CadB and its physiological functions in *Escherichia coli*. *Mol. Microbiol*. 2004; 51 (5): 1401–1412. DOI: 10.1046/j.1365-2958.2003.03913.x.
40. Goytia M., Hawel L., Dhulipala V.L., Joseph S.J., Read T.D., Shafer W.M., Characterization of a spermine/spermidine transportsystem reveals a novel DNA sequence duplication in *Neisseria gonorrhoeae*. *FEMS Microbiol. Lett*. 2015; 362 (16). DOI: 10.1093/femsle/fnv125.
41. Bachrach U. Naturally occurring polyamines: interaction with macromolecules. *Curr. Protein Pept. Sci*. 2005; 6 (6): 559–566.
42. Michael A.J. Polyamine function in archaea and bacteria. *J. Biol. Chem*. 2018; 293 (48): 18693–18701. DOI: 10.1074/jbc.TM118.005670.
43. Souzu H. Fluorescence polarization studies on *Escherichia coli* membrane stability and its relation to the resistance of the cell to freeze-thawing. II. Stabilization of the membranes by polyamines. *Biochim. Biophys. Acta*. 1986; 861 (2): 361–367.
44. Samartzidou H., Mehrazin M., Xu Z, Benedik M.J., Delcour A.H. Cadaverine inhibition of porin plays a role in cell survival at acidic pH/H. *J. Bacteriol*. 2003; 185 (1): 13–19.

45. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev*. 2003; 67 (4): 593–656.
46. Williams K. Interactions of polyamines with ion channels. *Biochem. J*. 1997; 325 (2): 289–297.
47. Katz A.M., Tolokh I.S., Pabit S.A., Baker N., Onufriev A.V., Pollack L. Spermine condenses DNA, but not RNA duplexes. *Biophys. J*. 2017; 112 (1): 22–30. DOI: 10.1016/j.bpj.2016.11.018.
48. Gevrekci A.Ö. The roles of polyamines in microorganisms. *World J. Microbiol. Biotechnol*. 2017; 33 (11): 204. DOI: 10.1007/s11274-017-2370-y.
49. Bae D.H., Lane D.J.R., Jansson P.J., Richardson D.R. The old and new biochemistry of polyamines. *Biochim. Biophys. Acta Gen. Subj*. 2018; 1862 (9): 2053–2068. DOI: 10.1016/j.bbagen.2018.06.004.
50. Igarashi K., Kashiwagi K. Polyamine modulon in *Escherichia coli*: genes involved in the stimulation of cell growth by polyamines. *J. Biochem*. 2006; 139 (1): 11–16. DOI: 10.1093/jb/mvj020.
51. Corella D., Guillén M., Hernández J.M., Hernández-Yago J. Effect of poliamine levels on the degradation of short-lived and long-lived proteins in cultured L-132 human lung cells. *Biochem. J*. 1998; 334 (2): 367–375.
52. Martin W.H.Jr, Pelczar M.J.Jr, Hansen P.A. Putrescine as a growth requirement for *Neisseria*. *Science*. 1952; 116 (3018): 483–484.
53. Elmros T., Burman L.G., Bloom G.D. Autolysis of *Neisseria gonorrhoeae*. *J. Bacteriol*. 1976; 126 (2): 969–976.
54. Anderson M.T., Byerly L., Apicella M.A., Seifert H.S. Seminal plasma promotes *Neisseria gonorrhoeae* aggregation and biofilm formation. *J. Bacteriol*. 2016; 198 (16): 2228–2235. DOI: 10.1128/JB.00165-16.
55. Patel C.N., Wortham B.W., Lines J.L., Fetherston J.D., Perry R.D., Oliveira M.A. Polyamines are essential for the formation of plague biofilm. *J. Bacteriol*. 2006; 188 (7): 2355–2363. DOI: 10.1128/JB.188.7.2355-2363.2006.
56. Li B., Maezato Y., Kim S.H., Kurihara S., Liang J., Michael A.J. Polyamine-independent growth and biofilm formation, and functional spermidine/spermine N-acetyltransferases in *Staphylococcus aureus* and *Enterococcus faecalis*. *Mol. Microbiol*. 2019; 111 (1): 159–175. DOI: 10.1111/mmi.14145.
57. Wotanis C.K., Brennan W.P., Angotti A.D., Villa E.A., Zayner J.P., Mozina A.N., Rutkovsky A.C., Sobe R.C., Bond W.G., Karatan E. Relative contributions of norspermidine synthesis and signaling pathways to the regulation of *Vibrio cholerae* biofilm formation. *PLoS One*. 2017; 12 (10). DOI: 10.1371/journal.pone.0186291.
58. Oppenheimer-Shaanan Y., Steinberg N., Kolodkin-Gal I. Small molecules are natural triggers for the disassembly of biofilms. *Trends Microbiol*. 2013; 21 (11): 594–601. DOI: 10.1016/j.tim.2013.08.005.
59. Anderson M.T., Byerly L., Apicella M.A., Seifert H.S. Seminal Plasma Promotes *Neisseria gonorrhoeae* Aggregation and Biofilm Formation. *J. Bacteriol*. 2016; 198 (16): 2228–2235. DOI: 10.1128/JB.00165-16.
60. Goytia M., Dhulipala V.L., Shafer W.M. Spermine impairs biofilm formation by *Neisseria gonorrhoeae*. *FEMS Microbiol. Lett*. 2013; 343 (1): 64–69. DOI: 10.1111/1574-6968.12130.
61. Anderson M.T., Dewenter L., Maier B., Seifert H.S. Seminal plasma initiates a *Neisseria gonorrhoeae* transmission state. *MBio*. 2014; 5 (2): e01004-13. DOI: 10.1128/mBio.01004-13.
62. O'Toole G.A., Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol*. 1998; 30 (2): 295–304.
63. Bachrach U. Metabolism and function of spermine and related polyamines. *Annu. Rev. Microbiol*. 1970; 24: 109–134.
64. Peng Y.C., Lu C., Li G., Eichenbaum Z., Lu C.D. Induction of the pho regulon and polyphosphate synthesis against spermine stress in *Pseudomonas aeruginosa*. *Mol. Microbiol*. 2017; 104 (6): 1037–1051. DOI: 10.1111/mmi.13678.
65. Yao X., Lu C.D. Characterization of *Staphylococcus aureus* responses to spermine stress. *Curr. Microbiol*. 2014; 69 (3): 394–403. DOI: 10.1007/s00284-014-0603-y.
66. Greiner L.L., Edwards J.L., Shao J., Rabinak C., Entz D., Apicella M.A. Biofilm Formation by *Neisseria gonorrhoeae*. *Infect. Immun*. 2005; 73 (4): 1964–1970. DOI: 10.1128/IAI.73.4.1964-1970.2005.
67. Kanjee U., Gutsche I., Alexopoulos E., Zhao B., El Bakkouri M., Thibault G., Liu K., Ramachandran S., Snider J., Pai E.F., Houry W.A. Linkage between the bacterial acid stress and stringent responses: the structure of the inducible lysine decarboxylase. *EMBO J*. 2011; 30 (5): 931–944. DOI: 10.1038/emboj.2011.5.
68. Kanjee U., Gutsche I., Ramachandran S., and Houry W.A. The enzymatic activities of the *Escherichia coli* basic aliphatic amino acid decarboxylases exhibit a pH zone of inhibition. *Biochemistry*. 2011; 50 (43): 9388–9398. DOI: 10.1021/bi201161k.
69. Nastri H.G., Algranati I.D. Effect of polyamines on plasmid mediated kanamycin resistance and kanamycin phosphotransferase gene expression in *Escherichia coli*. *Cell Mol. Biol*. 1996; 42 (5): 711–717.
70. Tkachenko A.G., Akhova A.V., Shumkov M.S., Nesterova L.Y. Polyamines reduce oxidative stress in *Escherichia coli* cells exposed to bactericidal antibiotics. *Res. Microbiol*. 2012; 163 (2): 83–91. DOI: 10.1016/j.resmic.2011.10.009.
71. Gong Z., Luna Y., Yu P., Fan H. Lactobacilli inactivate *Chlamydia trachomatis* through lactic acid but not H2O2. *PLoS One*. 2014; 9 (9): e107758. DOI: 10.1371/journal.pone.0107758.
72. Aldunate M., Tyssen D., Johnson A., Zakir T., Sonza S., Moench T. Vaginal concentrations of lactic acid potently inactivate HIV. *J. Antimicrob. Chemother*. 2013; 68 (9): 2015–2025. DOI: 10.1093/jac/dkt156.
73. Elmros T., Sandstrom G., Burman L. Autolysis of *Neisseria gonorrhoeae* relation between mechanical stability and viability. *Brit. J. Vener. Dis*. 1976; 52 (4): 246–249.
74. Chen K.C., Forsyth P.S., Buchanan T.M., Holmes K.K. Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *J. Clin. Invest*. 1979; 63 (5): 828–835. DOI: 10.1172/JCI109382.
75. Kanjee U., Houry W.A. Mechanisms of acid resistance in *Escherichia coli*. *Annu. Rev. Microbiol*. 2013; 67: 65–81. DOI: 10.1146/annurev-micro-092412-155708.

76. Zini A., O'Bryan M.K., Schlegel P.N. Nitric oxide synthase activity in human seminal plasma. *Urology*. 2001; 58 (1): 85–89.
77. Owen D.H., Katz D.F. A review of the physical and chemical properties of human semen and the formulation of a semen simulant. *J. Androl*. 2005; 26 (4): 459–469. DOI: 10.2164/jandrol.04104.
78. Mitchell C., Paul K., Agnew K., Gaussman R., Coombs R.W., Hitti J. Estimating volume of cervicovaginal secretions in cervicovaginal lavage fluid collected for measurement of genital HIV-1 RNA levels in women. *J. Clin. Microbiol*. 2011; 49 (2): 735–736. DOI: 10.1128/JCM.00991-10.
79. Richard H.T., Foster J.W. Acid resistance in *Escherichia coli*. *Adv. Appl. Microbiol*. 2003; 52: 167–186.
80. Fredricks D.N., Fiedler T.L., Marrazzo J.M. Molecular identification of bacteria associated with bacterial vaginosis. *N. Engl. J. Med*. 2005; 353 (18): 1899–1911. DOI: 10.1056/NEJMoa043802.
81. Hillier S., Marrazzo J.M., Holmes K.K. Bacterial vaginosis. In: Holmes K.K., Sparling P.A. (ed) Sexually transmitted diseases. 4th ed. New York: McGraw-Hill, 2008: 737–768.
82. Ravel J., Gajer P., Abdo Z., Schneider G.M., Koenig S.S., McCulle S.L., Karlebach S., Gorle R., Russell J., Tacket C.O., Brotman R.M., Davis C.C., Ault K., Peralta L., Forney L.J. Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci. USA*. 2011; 108 (l): 4680–4687. DOI: 10.1073/pnas.1002611107.
83. Srinivasan S., Hoffman N.G., Morgan M.T., Matsen F.A., Fiedler T.L., Hall R.W., Ross F.J., McCoy C.O., Bumgarner R., Marrazzo J.M., Fredricks D.N. Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. *PLoS One*. 2012; 7 (6): e37818. DOI: 10.1371/journal.pone.0037818.
84. Buve A., Jespers V., Crucitti T., Fichorova R.N. The vaginal microbiota and susceptibility to HIV. *AIDS*. 2014; 28 (16): 2333–2344.
85. Allsworth J.E., Peipert J.F. Severity of bacterial vaginosis and the risk of sexually transmitted infection. *Am. J. Obstet. Gynecol*. 2011; 205 (2): e1-6. DOI: 10.1016/j.ajog.2011.02.060.
86. Schwebke J.R., Desmond R. Risk factors for bacterial vaginosis in women at high risk for sexually transmitted diseases. *Sex Transm. Dis*. 2005; 32 (11): 654–658.
87. Taha T.E., Hoover D.R., Dallabetta G.A., Kumwenda N.I., Mtimavalye L.A., Yang L.P., Liomba G.N., Broadhead R.L., Chiphangwi J.D., Miotti P.G. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS*. 1998; 12 (13): 1699–1706.
88. Hummelen R., Fernandes A.D., Macklaim J.M., Dickson R.J., Changalucha J., Gloor G.B., Reid G. Deep sequencing of the vaginal microbiota of women with HIV. *PLoS One*. 2010; 5 (8): e12078. DOI: 10.1371/journal.pone.0012078.
89. Hillier S.L., Krohn M.A., Cassen E., Easterling T.R., Rabe L.K., Eschenbach D.A. The role of bacterial vaginosis and vaginal bacteria in amniotic fluid infection in women in preterm labor with intact fetal membranes. *Clin. Infect. Dis*. 1995; 20 (2): 276–278.
90. Haggerty C.L., Hillier S.L., Bass D.C., Ness R.B. Bacterial vaginosis and anaerobic bacteria are associated with endometritis. *Clin. Infect. Dis*. 2004; 39 (7): 990–995. DOI: 10.1086/423963.
91. Marrazzo J.M., Wiesenfeld H.C., Murray P.J., Busse B., Meyn L., Krohn M., Hillier S.L. Risk factors for cervicitis among women with bacterial vaginosis. *J. Infect. Dis*. 2006; 193 (5): 617–624. DOI: 10.1086/500149.
92. Srinivasan S., Morgan M.T., Fiedler T.L., Djukovic D., Hoffman N.G., Raftery D., Marrazzo J.M., Fredricks D.N. Metabolic signatures of bacterial vaginosis. *MBio*. 2015; 6 (2). DOI: 10.1016/j.ajog.2011.02.060.
93. Wolrath H., Forsum U., Larsson P.G., Borén H. Analysis of bacterial vaginosis-related amines in vaginal fluid by gas chromatography and mass spectrometry. *J. Clin. Microbiol*. 2001; 39 (11): 4026–4031. DOI: 10.1128/JCM.39.11.4026-4031.2001.
94. Chen K.C., Amsel R., Eschenbach D.A., Holmes K.K. Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. *J. Clin. Microbiol*. 2001; 39 (11): 4026–4031. DOI: 10.1093/infdis/145.3.337.
95. Bogdanov Y.A., Karpunina T.I., Nesterova L.Y., Akhova A.V. On the diagnostic significance of polyamines in semen of infertile men with asymptomatic inflammation. *Andrology and Genital Surgery*. 2013; 14 (3): 19–22 (in Russ.). DOI: 10.17650/2070-9781-2013-3-19-22.
96. Meng S.Y., Bennett G.N. Nucleotide sequence of the *Escherichia coli* cad operon: a system for neutralization of low extracellular pH. *J. Bacteriol*. 1992; 174 (8): 2659–2669.
97. Iyer R., Williams C., Miller C. Arginine-agmatine antiporter in extreme acid resistance in *Escherichia coli*. *J. Bacteriol*. 2003; 185 (22): 6556–6561.
98. Sobel J.D. Bacterial vaginosis. *Annu. Rev. Med*. 2000; 51: 349–356. DOI: 10.1146/annurev.med.51.1.349.
99. Giacomini G., Calcinai A., Moretti D., Cristofani R. Accuracy of cervical/vaginal cytology in the diagnosis of bacterial vaginosis. *Sex Transm. Dis*. 1998; 25 (1): 24–27.
100. Kwon D.H., Lu C.D. Polyamines induce resistance to cationic peptide, aminoglycoside, and quinolone antibiotics in *Pseudomonas aeruginosa* PAO1. *Antimicrob. Agents Chemother*. 2006; 50 (5): 1615–1622. DOI: 10.1128/JCM.00991-10.
101. Kwon D.H., Lu C.D. Polyamine effects on antibiotic susceptibility in bacteria. A*ntimicrob. Agents Chemother*. 2007; 51 (6): 2070–2077. DOI: 10.1128/AAC.01472-06.
102. Goytia M., Shafer W.M. Polyamines can increase resistance of *Neisseria gonorrhoeae* to mediators of the innate human host defense. *Infect. Immun*. 2010; 78 (7): 3187–3195. DOI: 10.1128/IAI.01301-09.

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