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Agr-typing of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolated from non-human primates

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SUMMARY

Staphylococcus aureus (*S. aureus*) is a microorganism that causes a great number of diseases in humans and animals, including sepsis, pneumonia, food toxico-infections, wound abscess, etc. Numerous studies on genotyping *S. aureus* strains isolated from humans, food and mastitis in cattle and small ruminants have been carried out. The lack of information on the genotyping of methicillin-susceptible *S. aureus* detected in monkeys served as a stimulus to conduct a similar research, since staphylococcal infections in the primates are widespread. The present study is devoted to molecular genetic testing of *S. aureus* isolated from different biological samples taken from monkeys and is based on typing of *agr* polymorphic locus which acts as a regulator of pathogenic gene expression. As a result of PCR analysis of 301 *S. aureus* isolates it was established that most of *S. aureus* belonged to *agr* IV (55%), and *agr* I (34%) was the second most group. Data resulting from the study differ from the results of other researchers published in literary sources, who performed typing of *Staphylococcus* isolated from people with *agr* I prevailing. In conducting the study, neither distinct correlation between microbial isolation source and *agr* complex groups, nor relationship between the diseases and *S. aureus* group specificity were detected. Prevalence ratio of each *agr* group is nearly similar in *S. aureus* isolated from rhesus macaques and crab-eating macaques. But in hamadryas baboons and green monkeys II and III groups of *agr* complex were not detected.

Key words: monkeys, *Staphylococcus aureus*, *agr* complex, *agr* groups and alleles.

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Agr-типирование метициллин-чувствительных *Staphylococcus aureus* (MSSA), выделенных у низших приматов

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РЕЗЮМЕ

Staphylococcus aureus (*S. aureus*) – микроорганизм, вызывающий большое количество заболеваний у человека и животных, включая сепсис, пневмонии, пищевые токсикоинфекции, нагноение ран и другие. По генотипированию штаммов *S. aureus*, выделенных у людей, из пищевых продуктов и при маститах у крупного и мелкого рогатого скота, проведено много исследований. Отсутствие данных по типированию метициллин-чувствительных *S. aureus*, обнаруженных у обезьян, побудило провести аналогичное исследование, поскольку инфекции стафилококковой природы у приматов широко распространены. Настоящая работа посвящена молекулярно-генетическому исследованию *S. aureus*, изолированных из разных биологических образцов от обезьян, на основе типирования полиморфного локуса *agr*, являющегося регулятором экспрессии генов патогенности. В результате исследования методом полимеразной цепной реакции 301 изолята *S. aureus* установлено, что большинство *S. aureus* относилось к группе *agr* IV (55%), на втором месте по распространенности оказался *agr* I (34%). Полученные в ходе исследования данные отличаются от опубликованных в литературных источниках результатов других исследователей, которые проводили типирование стафилококков, выделенных от людей, у которых превалирует *agr* I. При проведении работы не

выявлено четкой корреляции между источником выделения микроба и группами комплекса *agr*, а также не отмечена связь между заболеваниями и принадлежностью *S. aureus* к определенной группе. Соотношения распространенности каждой группы *agr* примерно одинаковы у *S. aureus*, изолированных у макаков-резусов и макаков яванских, но у павианов гамадрилов и мартышек зеленых II и III группы комплекса *agr* не обнаружены.

Ключевые слова: обезьяны, *Staphylococcus aureus*, комплекс *agr*, группы и аллели *agr*.

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INTRODUCTION

Infections caused by *Staphylococcus aureus* (*S. aureus)* play a significant role in veterinary science and medicine. Although *S. aureus* is a commensal microbe first of all, it can also cause a wide spectrum of diseases in humans and animals, which can significantly vary in their severity (skin infections, meningitis, endocarditis, osteomyelitis, bacteremia, toxic shock syndrome, food poisoning, mastitis, abscess, pneumonia, etc.). Being a zoonotic pathogen, it is responsible for infectious diseases characterized by septicemia and sepsis [1]. *S. aureus* has developed a complex regulatory network for control of virulence factors' production in order to survive and adapt to different ecological niches. One of the main functions of this interrelated network is the perception of different environmental signals and reaction by changing the production of virulence factors essential for survival in host, including cell-surface adhesins, extracellular enzymes and toxins. Accessory gene regulator (*agr*) system is considered to be the most investigated. It represents a gene cluster which regulates the expression of different housekeeping genes, various virulence factors and adhesive proteins recognizing the macromolecules of host cells and is also involved in quorum sensing. *Agr* locus is characterized by the polymorphism of its autoinducing peptide (AIP). Four main *agr* groups (I, II, III, IV) can be distinguished according to their variable regions upon which *agr*-typing is based [2–4]. It is established by the molecular genetic testing that *agr* groups are spread in different geographic zones, but at the same time detection of the prevailing groups may vary in each region [3].

There is little information available in literature on investigation of *S. aureus* isolated from animals. S. Monecke et al. provide data on *S. aureus* research in wild and exotic animal species, being kept in captivity [5]. In some cases, the molecular typing proved that human strains were transmitted to these animals. Many studies are devoted to the genetic peculiarities of *S. aureus* recovered in bovine mastitis [6–11]. Molecular genetic testing of *S. aureus* isolated from monkeys is performed at Adler monkey farm relatively recently [12–14]. No studies on *agr*-typing of *S. aureus* isolated from monkeys have been carried out abroad. Thus, given the important role of *agr* genes, this study was aimed at detection and identification of *agr* groups of *S. aureus* isolates recovered from different biological samples taken from monkeys.

MATERIALS AND METHODS

Recovery and identification of bacterial isolates. S. aureus isolates (301 isolates) recovered from different monkey species kept at Adler monkey farm are included in the study. *S. aureus* isolates were recovered from feces ($n = 62$) and nasal mucosa ($n = 32$) of live animals and from different organs of dead animals: lungs ($n = 101$), liver ($n = 36$), spleen ($n = 26$), kidneys ($n = 17$), lymph nodes ($n = 27$). For the purpose of the study, lungs were taken from monkeys died of pneumonia, and other organs – from monkeys with intestinal diseases.

Bacteriological and biochemical testing using standard methods were carried out for staphylococci isolation, as previously described [13, 14]. Identification was performed based on morphological, tinctorial and biochemical properties. Species identification was performed using commercial tests systems "Multimicrotests for biochemical identification of staphylococci (MMT C)" (NPO "Immunotex", Russia).

S. aureus strain DNA extraction. Total staphylococci DNA extraction was performed from bacterial suspensions prepared from day-old agar *S. aureus* cultures and suspended with 100 µl of NaCl solution using DNA-sorb-B reagent kit (InterLabService Ltd., Russia) according to the manufacturer's instruction.

S. aureus mecA gene detection with PCR. Polymerase chain reaction with hybridization-fluorescence detection was carried out using commercial AmpliSens® MRSA-screen-titre-FRT test system (InterLabService Ltd., Russia) in Rotor-Gene fluorescence-detecting thermocycler (USA) according to the attached instruction.

Agr complex allele detection and typing. Agr-typing of specific groups was performed using primers described in the scientific publications [4], and designed by the Evrogen (Russia) (Table 1).

Ready-to-use ScreenMix-HS amplification mixes (Evrogen, Russia) were used for multiplex PCR; final volume – 25 µl for the reaction. Amplification was performed in "Tercyc" thermocycler (Company DNA-Technology LLC, Russia) according to the following programme: predenaturation – at 95 °C for 5 min; denaturation – at 95 °C for 10 sec, annealing – at 50 °C for 10 sec, prolongation – at 72 °C for 20 sec (32 cycles) and final elongation – at 72 °C for 5 min.

Gel-electrophoresis. Amplification products were visualized with 1.2% agarose gel electrophoresis stained with ethidium bromide solution at voltage gradient of 90 V. Amplicon sizes were determined using 100–1200 base pair DNA marker (Evrogen, Russia).

Table 1
***Agr* locus genes and primers used in the analysis**

Таблица 1
Гены локуса *agr* и праймеры, использованные в исследовании

Gene/locus	5′–3′ Sequence	Scale, μmol	Amplicon (base pairs)
agr loci (*agrB*)	F: ATGCACATGGTGCACATGC	0.6	
agr I	R: GTCACAAGTACTATAAGCTGCGAT	0.4	441
agr II	R: TAT TAC TAA TTG AAA AGT GGC CAT AGC	0.4	575
agr III	R: GTAATGTAATAGCTTGTATAATAATACCCAG	0.4	323
agr IV	R: CGATAATGCCGTAATACCCG	0.4	659

RESULTS AND DISCUSSION

S. aureus isolates (301) were identified using phenotypic and biochemical tests. All tested cultures demonstrated hemoltical and lecithinase activity, fermented mannitol under anaerobic conditions, coagulated rabbit plasma. The results obtained completely matched (100%) the results of PCR with hybridization-fluorescence detection, which confirmed *Staphylococcus* type and showed the lack of mecA gene isolates in the genome, i.e. all the cultures were methicillin-susceptible (MSSA).

As a result of *agr*-typing, most of MSSA were attributed to *agr* group IV (55%). *Agr* I (34%) was the second frequent type. Detection frequency of other groups was far below (Table 2).

As Table 2 shows, *agr* complex allele IV dominated almost in all MSSA isolates recovered from the organs of dead monkeys, fecal and nasal cultures. Frequency of its detection varied from 41% in *S. aureus* isolated from kidneys to 61% in *S. aureus* isolated from the lymph nodes. However, no allele III was detected in 27 *S. aureus* isolates recovered from the lymph nodes.

Agr IV group also prevailed in MSSA isolated from monkeys of the following species: macaque (rhesus macaque, crab-eating macaque) and hamadryas baboons (Table 3). No *agr* complex groups II and III were detected in *S. aureus* recovered from hamadryas baboons and green monkeys.

As it is known, *S. aureus* is a commensal microorganism of mammal microbiota, but at the same time it expresses different pathogenicity factors becoming the cause of various hospital-acquired and community-acquired infections. Secretion of various surface cell proteins, toxins and adhesins is regulated by *agr* locus. Method of *S. aureus* classification on the basis of *agr*-typing was firstly used by P. Dufour et al. [2], who divided isolates of this microbe into four groups. This division is based on *agrC* gene encoding the autoinducing peptide receptor and *agrD* gene encoding the autoinducing peptide [2, 4]. *S. aureus* strains recovered from humans are characterized by four *agr* system allele groups connected with the genetic background and presence of the pathogenicity factors [15]. However, the relative distribution of *agr* groups in *S. aureus* isolates recovered from monkeys is still unknown.

Numerous studies have established that different *agr* groups may be associated with certain virulence factors and diseases caused by *S. aureus* [1, 15, 16]. It was determined that *S. aureus* isolates recovered from humans, belonging to *agr* I represent a group consisting of hospital-acquired and community-acquired isolates,

Table 2
***S. aureus* isolates belonging to *agr* groups according to the isolation source**

Таблица 2
Принадлежность изолятов *S. aureus* к *agr*-группам в зависимости от источника выделения

Source of isolation	Number of isolates according to *agr* groups, *n* (%)				Total
	agr I	*agr* II	*agr* III	*agr* IV	
Lung	35 (35%)	5 (5%)	3 (3%)	58 (57%)	101
Liver	12 (33%)	3 (8%)	2 (6%)	19 (53%)	36
Spleen	8 (31%)	1 (4%)	2 (8%)	15 (57%)	26
Kidney	8 (47%)	1 (6%)	1 (6%)	7 (41%)	17
Lymph node	11 (35%)	2 (4%)	–	14 (61%)	27
Nasal swab	13 (41%)	2 (6%)	1 (3%)	16 (50%)	32
Feces	16 (26%)	8 (13%)	3 (5%)	35 (56%)	62
Total	103 (34%)	22 (7%)	12 (4%)	164 (55%)	301

Table 3
***Agr* group distribution in *S. aureus* isolates obtained from different monkey species**

Таблица 3
Распространение *agr*-групп у изолятов *S. aureus*, полученных от различных видов обезьян

Monkey species	Number of isolates according to *agr* groups				Total
	agr I	*agr* II	*agr* III	*agr* IV	
Rhesus macaque	39	7	3	64	113
Crab-eating macaque	32	11	7	55	105
Southern pig-tailed macaque	3	2	1	4	10
Green monkey	4	–	–	5	9
Anubis baboon	5	1	1	8	15
Hamadryas baboons	15	–	–	25	40
Bear macaque	2	1	–	–	3
Capuchin monkey	1	–	–	2	3
Patas monkey	2	–	–	1	3
Total	103	22	12	164	301

rare sporadic strains; *agr* II and *agr* III groups are mainly hospital-acquired epidemiologic clones; *agr* IV – is a rare group, resembling *agr* I [17]. Staphylococci belonging to *agr* III group are associated with invasive diseases, such as bacteremia [3]. *S. aureus* comprising *agr* I groups are capable to penetrate into epithelial cells and cause mastitis in cows and sheep [9].

Studies of foreign and domestic researches demonstrate that allele I is prevailing among *S. aureus* isolates recovered from humans in different geographic regions [4, 5, 16]. In some research works aimed at investigation of *S. aureus* isolated in cow mastitis *agr* I was also dominating [3, 5, 7, 11], but in other studies *agr* II and *agr* III took the first place [8]. *Agr* IV group in mastitis of cattle and small ruminants was found in very rare cases or was not found at all [5, 7, 8, 11]. It is described in works of L. M. De Almeida et al. [9] that alleles I and II were detected in *S. aureus* in sheep milk and sheep mastitis. Data on distribution of *agr* groups in *S. aureus* isolated at pig farms were also various. Indeed, in such cases some authors [18] report on detection of only *agr* I in *S. aureus*, but the others – only of *agr* IV group [1]. It is probable that the spread of the given alleles of gene regulating complex in *S. aureus* isolated from animals, is connected with the geographical region, as well as in humans.

In this investigation, most of MSSA isolates recovered from monkeys were attributed to *agr* IV group (55%), and *agr* I (34%) was the second most group. *Agr* II alleles were detected in 7% and *agr* III – in 4% of tested isolates. *Agr* IV group dominated in staphylococci isolated from monkey lungs affected with pneumonia and in those isolated from the organs affected by intestinal infections. According to the research results it is established that the isolates colonizing monkey nasal mucosa and recovered from kidneys equally belong to *agr* I and *agr* IV. Allele IV of the tested complex is 20–30% more likely to be found in *S. aureus* detected in other organs and fecal samples. It is also mentioned in the study that the prevalence ratio of each *S. aureus agr* group is nearly similar, in case with rhesus macaques and crab-eating macaques. But no alleles II and III were detected among all the tested hamadryas baboons and green monkeys. Data obtained in the course of the work differ from those concerning the distribution of *agr* complex alleles in *S. aureus* strains isolated from humans, where *S. aureus agr* I groups are prevailing [2–4, 15].

The recent study suggests further molecular-genetic examination with *spa*- and *coa*-typing of isolates in order to understand the epidemiology of *S. aureus* in monkeys at monkey farm and compare the obtained results with the molecular characteristics of epidemiological clones recovered from humans.

CONCLUSIONS

1. *Agr* IV group dominated in *S. aureus* isolates recovered from monkeys.

2. No correlation between the source of microbe isolation and *S. aureus* distribution to a certain *agr* group was detected.

3. No *agr* complex alleles II and III were detected in *S. aureus* isolates recovered from hamadryas baboons and green monkeys.

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