

terobacteriaceae species from mosquito gut microflora on inhibiting the development of *Plasmodium* [4], the aim of these investigations was to characterize the predominant *Enterobacteriaceae* isolates in midgut microflora of *Anopheles* mosquitoes from the different regions of Armenia, collected during the summer of 2011.

Materials and methods. Mosquitoes collection and processing. The mosquitoes' samples were collected from five locations: Lori, Syunik and Vayots dzor regions in summer, 2011 (Figure).

For the isolation of bacteria on *Anopheles* mosquitoes, the 5 pools mosquitoes of 10 mosquitoes each were homogenized in 1.5 ml of phosphate buffered saline (PBS), resuspended it again in 1,5 ml Luria–Bertani (LB) medium (10g tryptone; 5g yeast extract; 10g NaCl per liter; pH 7.5).

During the midgut bacterial investigations, the mosquitoes were sterilized 3sec. in 95% ethanol, and dissected under the microscope. The midgut extracts in 100 µl sterile PBS solution were vortexed for 2 min. The debris was removed by low-speed centrifugation (700xg, 5min) and the supernatant was serially diluted in PBS. The number of colony forming units (CFU) of *Enterobacteriaceae* was determined by colony count of dilution series from fresh extracts applied to MacConkey agar plates with further use of the selective media, antibiotic discs and conventional biochemical testing [13,14]. Gram staining and motility for the identification of *Enterobacteriaceae* species was also performed, and the **Enterobacteriaceae** on *An.* mosquitoes and its midgut microflora were investigated utilizing a culture dependent pathway.

Investigations on bacterial growth. Bacteria were grown in LB medium at pH 7.0, 9.0, 9.5, 10.0 and 10.5 as described earlier. The pH of medium was adjusted by using NaOH or HCl. In order to assess the role of different carbon sources in the bacterial growth, the cells have been grown in M9 medium with addition of Glucose, Lactose, D-Mannitol, D-Sorbitol, (+)-Xylose, L-(+)-Arabinose or Dulcitol [13].

The susceptibility to the next antibiotics: tetracycline 15 µg/ml (Oxoid), doxycycline 15 µg/ml (Biomerieux), amoxicillin 25 µg/ml (Biomerieux), ampicillin 35 µg/ml (Biomerieux), kanamycin 50 µg/ml (Oxoid), gentamicin 50 µg/ml (Oxoid), chloramphenicol 30 µg/ml (Oxoid), streptomycin 50µg/ml (Oxoid) were tested during the investigations. The cells were plated on LB-agar with corresponding antibiotics and inspected for growth after incubation for 24 h at 37°C.

Statistical analysis was performed using the CHITEST (null hypothesis). The probability of $p < 0.05$ was to reject the null hypothesis.

Results and Discussion. The *Klebsiella*, *Edwardsiella* and *Escherichia* species were revealed on mosquitoes and its midgut microflora during our investigations (Table 1).



Map of Armenia; ★- mosquitoes collection location

According to literature data, *Enterobacteriaceae* species, presented in mosquito midgut, may be pointed out as pathogens of mosquitoes and may negotiate the ability of *Plasmodium* to establish infection with different mechanisms [3]. Mosquitoes, treated with antibiotics, became more susceptible to *Plasmodium* infection [4]. The presence of *Klebsiella* and *Edwardsiella* species in midgut microflora of mosquitoes, collected in Armenian regions, possibly influences on *Plasmodium* infection here. At the same time, *Escherichia* found only on mosquitoes, which could be result of environmental contamination (Table 1).

Table 1
Predominant *Enterobacteriaceae* isolates identified during the investigations on Anaphales mosquito, collected from Armenian regions during the summer of 2011

Bacterial sources	<i>Enterobacteriaceae</i> isolates				
	Tashir (LORI region) August, 2011	Fioletovo (LORI region) August, 2011	Sarnanunk (Syunik region) June, 2011	Gexi (Syunik region) June, 2011	Vayk (Vayots Dzor region) June, 2011
On mosquitoes	<i>Edwardsiella</i> <i>Escherichia</i>	<i>Klebsiella</i> <i>Escherichia</i>	<i>Klebsiella</i>	<i>Klebsiella</i> , <i>Edwardsiella</i>	<i>Edwardsiella</i>
In midgut microflora	<i>Edwardsiella</i>	<i>Klebsiella</i>	<i>Klebsiella</i>	<i>Klebsiella</i>	<i>Edwardsiella</i>

The anterior half of the larval mosquito midgut, in opposite of most other organisms, has a 10.5-11.5 luminal pH, that probably is conditioned by presence of bicarbonate/carbonate in gut lumen [15]. Taking into account this data, the growth of revealed *Enterobacteriaceae* isolates at pH 7.0, 9.0, 9.5, 10.0 and 10.5 of growth medium during our future investigations were studied. As have

shown the results of these investigations the isolates keep their growth abilities in pH 10.0 of LB medium. 17 % of these isolates was resistance two or more classes of investigated antibiotics.

during the last years (Table 2). In spite of there is not data on human infection in different locations of Armenia during the 2011, we assume that the increasing number of infections might be conditioned by the high number of

Table 2
Results of prophylactic examination of children at the age of 0-14 years old in Armenia*

Diseases	2006	2007	2008	2009	2010
Infection and parasitic diseases	36552	28386	28791	28538	32776
Blood diseases and other hematogenic disturbances	4631	4765	4847	5303	5372
Digestive organs disorders	17137	15442	15289	14899	16268
Urogenital disorders	3728	3953	4484	4576	5115
Skin infection and underskin fat diseases	9955	10020	11179	12657	14065
Osteo-muscular and connective tissue disorders	1469	1295	1227	1240	1466
Number of diseases per 100 000 children - total	39713.6	41001.6	43785.1	51379.9	51460.9

* <http://www.armstat.am/file/doc/99466648.pdf> (*Yearbook of Armenia-2011*)

opportunistic pathogens in ecosystem.

Malaria infections remain one of impactful vector-borne disease over the world. Current investigations necessitate the significance of studies on microbiology of mosquitoes and soil and animals surrounding of mosquitoes in malaria-less regions.

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**Characterization of *Enterobacteriaceae* Isolated from *Anopheles*
Mosquitoes in Armenia**

The effect of various types of Enterobacteries which were isolated from the intestinal microflora of mosquitoes, on the growth and development of Plasmodium was examined. Samples of intestinal microflora of the mosquitoes were collected from different regions of Armenia: Lori, Syunik and Vayots Dzor.

It was found that the presence of samples of Klebsiella, Escherichia and Edwardsiella in the intestinal microflora of the mosquitoes, inhibit the growth and the development of plasmodium infections and, thus, prevent the development of malaria.

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**Հայաստանում հավաքած *Anopheles* տեսակի մեղիների աղիքային
մանրէների մեկուսացումը և բնութագիրը**

Հետազոտվել է մալարիայի մեղի աղիքային միկրոֆլորայից առանձնացրած տարբեր տեսակի էնտերոմանրէների ազդեցությունը պլազմոդիաների աճի և զարգացման վրա: Մալարիայի մեղի միկրոֆլորայի նմուշները վերցված են Հայաստանի տարբեր մարզերից՝ Լոռիից, Սյունիքից, Վայոց Ձորից:

Պարզվել է, որ մալարիայի մեղի աղիքային միկրոֆլորայում առկա *Klebsiella*, *Escherichia* *Edwardsiella* էնտերոմանրէները խոչընդոտում են պլազմոդիաների ազդեցությունից առաջացած վարակի աճին և զարգացմանը, այդ թվում նաև մալարիայի:

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**Выделение и характеристика кишечных бактерий комаров рода
Anopheles, собранных в Армении**

Исследовано влияние различных видов энтеробактерий, изолированных из микрофлоры кишечника малярийного комара, на рост и развитие плазмодий. Пробы микрофлоры кишечника малярийного комара отбирались в различных регионах Армении: Лори, Сюнике и Вайоц Дзоре.

Выяснилось, что наличие в микрофлоре кишечника малярийного комара *Klebsiella*, *Escherichia* *Edwardsiella* ингибируют рост и развитие плазмодийных инфекций и следовательно препятствуют развитию малярии.

