The antibiotic use by pregnant mice was a reason of lung pathology of their offspring

Tatiana Khomyakova¹, Vasilii Kozlovsky², Galina Kozlovskaya¹, Aminat Magomedova¹, and Yuri Khomyakov³

¹FGBNU “SRI of human morphology”, Thyurupa st., 3, Moscow, Russia,
²FGBNU “Afanasyev institute of fur farming and rabbit breeding”. Moscovskaya oblast, Russia,
³FKUZ “Antiplague center of Rospotrebnadzor”, Musorgskogo st., 4, Moscow, Russia

E-mail: tatkhom@yandex.ru

Abstract. The inappropriate antibiotic usage in human and animal treating is a main reason of the antibiotic resistance, spreading wider and wider all over the world. It was proved that the use of antibiotic induces the changes in microbiota profile as well as the protective properties of the intestinal mucosal barrier, determining the level of the bacterial translocation to the distant loci of the body. Pneumonia in newborns is one of the severe pathologies that cause increased neonatal morbidity and mortality. During the newborn period, pneumonia develops much more often than in other age periods. It is considered that main reason of respiratory adaptation disorders and newborns’ pneumonia is the intrauterine infection and the development of the inflammatory process. In animal husbandry we can see that a non-specific bronchopneumonia is one of the main causes of mass mortality of young cattle. There is no any confirmed reason of the growing level of the mortality of cattle. Here we present the results of our investigation which allow suggesting a role of antibiotic use by pregnant females as a basis for the following bacterial translocation to the fetus and a non-specific bronchopneumonia.

1. Introduction

The antibiotic use is the main approach in veterinary for the treating of all infection diseases. However, in the last decades the antibiotic use is confirmed to be the reason of several serious problems in the whole world. First of all, antibiotic use in farm animals can select for multidrug-resistant bacterial strains in animals as well as in the environment. The randomized controlled longitudinal field trial has confirmed that the antibiotic use in cattle can select for Salmonella enterica, which is considered a serious threat [1]. There is a growing global concern about antimicrobial resistance among enteric pathogens due to an observed increase of cephalosporin- and fluoroquinolone-resistant Salmonella in humans [2]. Increasing ceftriaxone and ciprofloxacin resistance was reported in Salmonella isolates recovered from human cases. Both chlorotetracycline and the third-generation cephalosporin, ceftiofur, are antibiotics used for the treatment and control of bovine respiratory diseases in feedlot cattle. It as shown that the prevalence of salmonella decreased immediately during and following ceftiofur and treatment ; however, the proportion of multidrug resistant (MDR) salmonella also increased [3] The use of ceftiofur in food animals has increased due to its high effectiveness, convenient formulations, short withholding, and increased label indications.
This increased use has been accompanied by a parallel increase in the prevalence of ceftiofur resistant (cefR) enteric bacteria in food animal populations.

Antibiotic resistance is a significant public health concern, that’s why in 2012 FDA has issued special rules for cephalosporin use in food animals [4]. In the U.S. resistance to 3rd-generation of cephalosporin is most often mediated by a blaCMY-2 gene that is harboured by IncA/C plasmids in enteric bacteria. [5] At least 97% of dairy herds in Washington State were positive for blaCMY-2 plasmid-bearing E. coli. The percentage of Salmonella sp. with ceftiofur resistance isolated from cattle increased from 0% in 1997 to 21.6% in 2005 [6]. The acquisition of antimicrobial resistance by pathogens has challenged our ability to treat infections and the emergence of antimicrobial resistant microorganisms represents a serious contemporary threat to public health and medicine [7].

One more problem of course is the presence of the antibiotic in the milk and meat, which are used for the human consumption. Even the small concentrations of them can be the reason of a serious pathology in adult and children. In our experiments we had previously found that the changes of gut microbiota structure in pregnant mice led to the similar shifts in their offspring. Later these results were confirmed by other authors [8, 9]. Except of it we have found that the long-term and aggressive chemical influence to the gut barrier leads not only to the pathobiome formation but to the bacterial translocation to the lungs. For example, we have shown that Klebsiella pneumonia, which was the reason of lethal pneumonia in HIV-infected patients, had the gut microbiota as a source of its origin at least in 50 % of all cases of mortality.

The aim of the work was to find if the antibiotic –induced changes of the gut microbiota lead to the similar changes in pregnant mice and their offspring and determines what species of bacteria can get the lungs of the pups inducing the lung pathology. Here we used the outbred mice because in our previous experiments the offspring of the inbred mice after the similar manipulations with the pregnant mice was born non-viable or was subjected to intrauterine resorption. Except of it, humans are an outbred species characterized by considerable heterozygosity and genetic diversity, so a lot of scientists confirm the better results of the use of outbred mice for investigations [10, 11].

2. Materials and methods

White outbred mature female mice ICR (CD 1) with a body weight of 18-20 g were used in the work. All manipulations with animals were carried out in accordance with the requirements of international and Russian legislation governing the “Rules for the handling of laboratory animals”. The mice received the standard feeding and water mode. Microbiological methods were used to assess dysbiosis. The severity of translocation was assessed by the determining the total bacterial load in the lungs of the offspring of mice with dysbiosis induced by the subcutaneous injection of an antibiotic cefotaxime in a dose equivalent to the therapeutic. The right caudal lobe was used as a standard sample for the bacterial estimation.

The species identification of bacteria was determined using a Multiskan FC microbiological analyzer (Thermo FS). The pieces of the left lung were taken for the histology. They were fixated in Buena fixating solution with the following washing in 70% ethanol and standard preparation of histological slides and hematoxylin and eosin staining.

The female mice were caged with male mice in a ratio of 5 to 1 for five days and then makes were taken away and females were distributed to groups. One of them was intact, and the other was experimental group. On the 15th day of the gestation the mice of the second group were injected with cefotaxime once a day during 5 days.

The feces of all mice were suspended and the lactobacilli level was determined. The dysbiosis was considered confirmed if the level of lactobacilli was reduced by three orders of magnitude compared with the control one. After giving birth, the mice were kept under normal conditions, on standard food and water. The mice formed nests and fed the offspring. One month after giving birth, after switching the offspring to independent feeding, adult mice were removed from the experiment by cervical translocation. For microbiological examination, feces and the caudal lobe of the right lung were taken.
21 days after that, the offspring were taken away from the experiment; samples were taken for microbiological research in the same way as described above.

3. Results
Injection of a cephalosporin drug did not affect the total number (32 heads in a control group and 48 heads in an experimental group) and the viability of the offspring. To the end of the suckling period, all the offspring did not differ in weight and appearance, the mice moved actively, consumed food and water. The injections of cefotaxime led to the development of a dysbiotic state of the large intestine in adult females, which manifested itself in an increase in the number of opportunistic bacteria and fungi and a decrease in lactobacilli in feces. These shifts were kept in the experimental group till the end of the study. Aerobic bacteria were not isolated out of the homogenate of the caudal lobe of the lungs of adult mice of both groups.

After 21 days of self-feeding, the offspring of experimental groups significantly differed in appearance: the mice had dull eyes, tousled hair, moved little and did not take food well. In the offspring of group 1, the level of lactobacilli in the feces was reduced compared with a control group (3.2-4.1 x 10^2 CFU/g and 4.5-6.8 x 10^5 CFU/g correspondingly). The number of opportunistic bacteria increased in the feces of the mice of the experimental group. The bacterial load in the lungs increased in all cases in the offspring of the mice received cefotaxime. The level of the aerobic microbes growing at the simple Luria- Bertani nutrient medium was from 3 to 60 thousands per the standard sample, while in the control group the bacteria was found only in one case and there was 2x10^3 CFU per the standard sample.

The species identification revealed the bacteria specific for the mice colon microbiota (Citrobacter rodentium, Bacillus megaterium, Enterococcus hirae, Acinetobacter Iwoffii, Staphylococcus warnery and Lactococcus garvieae) were isolated. In two cases the strains of Lactobacillus murinus were isolated out of lungs of the offspring that confirm that the gut is the origin of the spreading of bacteria. In one case three different strains of bacteria were isolated out of the same animal: Lactococcus garvieae, Citrobacter rodentium and Lactobacillus murinus. We should remember that translocation out of the gut touches both aerobic and anaerobic bacteria as well as non-cultivated bugs, so the whole process of bacterial migration should be much more complex and aggressive.

The histological analysis has shown the decreased airiness of the lungs with the foci of interstitial pneumonia in the lungs of the offspring of the mice of the experimental group.

4. Discussion
It is well known that the bacterial translocation from the gut to the maternal blood stream and from there to other organ systems is increased during pregnancy and lactation [12]. The possibility of infecting the fetus in utero is being discussed during the last decades. This is highly possible that bacteria are going through the amniotic fluid. For example, intrauterine infection is considered the most common cause of spontaneous preterm birth [13].

In their experiments Jiménez et al. [14] orally inoculated pregnant mice with genetically labeled Enterococcus that had been previously isolated from the breast milk of a healthy woman. The labeled bacteria were retrieved after term cesarean section delivery of pups from the internal meconium. However, labeled bacteria were not detected in the pups of control animals that had not been inoculated. So it is possible to say that the gastrointestinal tract is a source of the pathogens for in utero infecting.

Except of it D. D. Nyangahu et all (2015) described the potential mechanisms through which maternal gut microbes during pregnancy impact infant immunity. They are sure that immune development in the fetus begins prior to delivery and is probably driven by translocation of microbiota or their metabolites from the maternal gut to the maternal–fetal unit or other mucosal surfaces [15].

We don’t insist that described process is the only way of the intrauterine infection and the development of the inflammatory process in lungs, but we are sure that the first source of the origin of
pathogens is the mothers gut and any reason induced the bacterial translocation can be considered as a potentially dangerous for the fetus.

5. Conclusion
The intrauterine infection of the fetus and the subsequent development of diseases of the bronchopulmonary system may be the result of bacterial translocation caused by a violation of the mucosal intestinal barrier and dysbiotic changes in the microbiome. These changes can be induced by the use of antibiotics by the mother in the last trimester of pregnancy.

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