ANTIBIOTICS AS AN ECOLOGICAL FACTOR

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ABSTRACT

Uncontrolled using of antibiotic in one geographic area for therapy, prophylaxis, animals' growth promotion and food preservation, have unforeseeable consequences. Antibiotics act as an ecological factor; eliminate susceptible and favor resistant species and strains from the microecologica ecosystems of human and animal organisms, which form commensals and opportunistic pathogens. This selective pressure has consequences such as complication of therapy after infection by resistant strains, disturbing balance of microbial communities and spreading the resistant bacteria and their genes worldwide.

The aim of our study was to test antibiotic susceptibility of nonpathogenic and pathogenic bacterial strains from genus Enterobacteriaceae (E.coli and Salmonella sp.), isolated from different specimens. The results show very high percent of resistant strains to the most commonly used antibiotics (penicillin, ampicillin, tetracycline, erythromycin, etc.).

We also discovered that antibiotics have mutagenic effect to bacteria, because strains treated with low concentration of amoxicillin and penicillin in laboratory, show significant level of increasing the resistance to other antibiotics. Our results show that antibiotics are strong ecological factor that has influence on animals and people by changing characteristic of microbial communities and whole life of ecosystems.

KEYWORDS: *resistance, antibiotics, enterobacteria*

1. INTRODUCTION

The great number of studies that engage in problem of antibiotic resistance of human pathogenic bacteria has opened many questions about usage and role of antibiotic in agriculture and medicine. Main reason for phenomenon of resistance is inadequate use of antibiotics. Scientist generally agree with opinion that bacterial resistance is not only result of antibiotics usage in therapeutic purposes, but also in other fields, especially in agriculture.

Usage of antibiotics as an additive in feed, for prophylaxis and growth promotion, has had impact on spreading of resistance. There are a lot of evidences that spreading of antibiotics resistance is connected with commensal bacteria, especially with commensals of gastrointestinal tract (7). Antibiotics act as an ecological factor; eliminate susceptible and favour resistant species and strains from the micro ecological ecosystems of human and animal organisms, which form commensals (non-pathogenic bacteria) and opportunistic pathogens. So, intestinal micro flora is carrier of resistance genes to many antibiotics and these bacteria reach into milk, meat, water and other mediums by fecal contamination. According to this, intestinal bacteria are one of the reservoirs of resistance genes and they are able to transmit these genes to sensitive pathogenic bacteria.

Quantity of antibiotics that are used in the world decrease every day, and one half is produced for human usage.. Bacteria from Enterobacteriaceae in animal gastrointestinal tract are especially exposed to antibiotics, because this therapeutics are being added into water and food. Also, these bacteria, when expire by feces or urine in environment, could be exposed to antibiotics taht are excreted in their active form from body and could cumulate in environment (2, 7). For example, during fish feeding has been estimated that 70-80% of antibiotics used for therapy of fish, could be detected in the water sediment. About $25x10^6$ kilograms of antibiotics are used every year for prevention infectious diseases and promotion of animal growth. In European Union, 3000 tones per year are used by veterinarian for therapy. In the US this quantity is about 8.500-11.200 tones (3). One part of these antibiotics is degraded in intestinal tract, but other is excreted in environment.

Because of this disturbing situation we examined resistance of strains of *E. coli* and *Salmonella typhimurium and S. enteritidis* isolated from different speciment. Also, we examined changes in sensitivity of some strains when these are exposed to low doses of antibiotics.

2. MATERIALS AND METHODS

Strains of *S. enteritidis*, *S. typhimurium* and *E. coli* were isolated from different materials (feed, animal feces, pigs, poultry and human infants) during routine bacteriological control. For isolation and subcultivation of test strains we used Endo agar, brilliant green agar, MacConkey agar, and nutrient broth. Identification of strains was performed by standard bacteriological methods.

Test strains of *S. enteritidis* and *E. coli* have been isolated from feed and feces. Strains were inoculated in nutrient broth and incubated at 37^0C 24 hours. After this period all strains were tested by Kirby Beyer method. One milliliter of culture suspensions were spread on Muller Hinton plate. Susceptibility of strains was tested to penicillin, streptomycin, ampicillin, neomycin, erythromycin, tetracycline, cephalexin, chloramphenicole, gentamicin, and linkomycin.

Susceptibility of two test strains of *E. coli* and *S. enteritidis* was examined in vitro before and after incubation with low doses of antibiotics. Selected strains of *S. enteritidis* and *E. coli* were suspended in 1% peptone water and physiological saline, with dense of 8 MC. In these suspensions was added low concentration of penicillin or amoxicillin, they were stored at 37^0C 48 h and than tasted to antibiotic susceptibility. The experiment was repeated four times. Susceptibility of strains was detected by Kirby Beyer method.

3. RESULTS AND DISCUSSION

Results of susceptibility of *E. coli, S. entritidis* and *S. tiphimurium* are showed in table 1 and 2.

Table 1. *Resistance (%) of S.enteritidis and S.typhmurium isolated from different animal speciments*

Terapeutics	*S.enteritidis*			*S.typhmurium*		
	Poultry	Feed	Animals' feces	Poultry	Pigs	Feed
penicilin	60,15	82,14	60,12	69,14	98,18	60,12
streptomicin	50,6	49,9	52,11	49,2	45,8	50,00
ampicillin	75,16	75,15	88,18	75,16	78,17	75,00
neomicin	82,17	74,14	89,18	72,14	65,13	76,16
erythromycin	100	90,19	86,18	82,17	98,19	88,17
tetracycline	100	78,17	76,16	100,00	98,19	75,00
cephalexin	62,14	64,13	50,00	51,11	50,00	62,12
chloramphenicol	90,19	88,18	99,19	60,12	75,00	74,14
gentamicin	43,7	39,6	25,5	49,95	49,99	39,70
linkomicin	100,0	99,19	100,00	88,18	78,16	99,19

Table 2. *Resistance (%) of E. coli isolated from diifferent speciments*

Terapeutics	Poultry	Pigs	Feed	Animals' feces
penicilin	69,14	89,18	50,00	79,80
streptomicin	60,12	44,90	71,14	59,11
ampicillin	98,18	77,72	65,13	77,17
neomicin	67,13	68,17	50,00	62,12
erythromycin	98,19	99,19	91,18	98,19
tetracycline	98,19	75,00	74,14	81,17
cephalexin	26,00	49,90	45,80	25,00
chloramphenicol	88,18	98,29	76,00	99,00
gentamicin	49,20	47,35	49,00	38,00
linkomicin	78,17	88,25	76,16	77,17

Our investigation showed high percent of resistant strains from animal, food and feces and can be explained by prolonged usage of these antibiotics on animal farms. This opinion is supported by studies of geneticists that showed that the same resistance genes can be found in the bacteria of animals and humans (12). Sunde et al. (10) was examined about 1200 strains of *E. coli* from health and ill pigs and found that the least resistance for an antibiotic was 100%, and that the most resistance was to streptomycin, sulfonamide and tetracycline. Similar results of *E. coli, Haemophylus influenzae* and *S.aureus* resistance was obtained from volunteers (4). Our experiment showed that 44,90 - 71,4% of tested isolates was resistant to streptomycin. Our results are in correlation whit these findings. Resistance of *S. tiphimirium*, *S. enteritidis* and *E. coli* isolated from human infants is shown in table 3.

Table 3. *Resistance of S.enteritidis, S. typhimurium i E.coli isolated from human infants*

antibiotics	***S.enteritidis***	***S.typhimurium***	***E.coli***
ampicillin	3,2	75	16,7
cephalexin	15,9	32,9	33,3
cefotaxime	0	0	16,7
gentamicin	0	0,9	0,9
trimetoprim	1,8	1,8	2
norfloxacin	0	0	0
ciprofloxacin	0	0	0

The low percent of resistant strains from human infants is probably resulting from infection with strains that are highly sensitive and that could not infect adults that use some of these antibiotics time to time. Some of these strains are commensal and opportunistic pathogens, especially to infants, and because there is no time to develop resistance they are still sensitive. Our opinion is that during the life, some of these bacteria, persisting as commensal, have a great chance to develop resistance or to gain resistance genes from other bacteria that will reach to gastrointestinal tract. Also, antibiotics used in this test are less common used.

Results of susceptibility of S. enteritidis and *E. coli* strains before and after exposure to low doses of antibiotics are showed in table 4 and 5.

Table 4. *Susceptibility of S. enteritidis stored in 1% peptone water and physiological saline with antibiotics*

Terapeutics	1% peptone water			Physiological saline		
Antibiotics	**control**	**penicilin**	**amoxicillin**	**control**	**penicillin**	**amoxicillin**
Penicillin	I	R	R	I	R	R
Streptomycin	S	R	I	S	I	R
Ampicillin	I	I	I	I	I	I
Neomycin	S	R	R	S	R	R
Erythromycin	I	I	I	I	R	R
tetracyckine	I	R	R	R	R	R
Cefalexin	I	I	I	I	I	I
Chloramphenicol	S	I	S	S	I	S
Gentamicin	I	I	I	I	I	I
Linkomycin	R	R	R	R	R	R

R- *resistant*, I- *intermediary susceptible* S- *susceptible*

Tested strains showed different susceptibility to used therapeutics. Resistance to linkomycin was detected before and after exposure to low doses of antibiotics. Supplementation of low doses of penicillin did not have any effect to change resistance of tested strain to penicillin, cephalexin, erythromycin and tetracycline. These results can be explained with fact that these four antibiotics are wide used in veterinarian medicine. Amoxicillin, added in physiological saline had effect on change of resistance to erythromycin and tetracycline. Resistance to erythromycin and tetracycline can be explained with their frequent usage to prevention and therapy of animals' infectious diseases from our area. Susceptibility of strains to chloramphenicol decreased after supplementation of penicillin. Susceptibility of strains stored in 1% peptone water was considerably greater to streptomycin and gentamicin than susceptibility of strains stored in the physiological saline.

Table 5. *Susceptibility of E. Coli strains stored in 1% peptone water and and physiological saline with antibiotics*

Terapeutics	1% peptone water			Physiological saline		
Antibiotics	**control**	**penicilin**	**amoxicillin**	**control**	**penicillin**	**amoxicillin**
Penicillin	R	R	I	R	R	R
Streptomycin	S	R	I	S	I	S
Ampicillin	I	I	I	I	R	R
Neomycin	I	I	I	I	R	R
Erythromycin	I	R	R	R	R	R
tetracyckine	I	R	R	R	R	R
Cefalexin	R	R	I	I	R	I
Chloramphenicol	S	S	S	S	I	S
Gentamicin	I	R	I	S	I	I
Linkomycin	R	R	R	R	R	R

R- *resistant*, I- *intermediary susceptible* S- *susceptible*

All tested *E. coli* strains were resistant to linkomycine. Strains from both medium have showed lower susceptibility to amoxicillin, erythromycin, caephalexin, and gentamicin. This susceptibility was not changed after exposure of test strains to low doses of antibiotics, except when they were treated with erythromycin. Streptomycin and neomycin showed the most similar effects to change resistance pattern of tested strains. These results can be consequence of their common origin (they are product of Streptomycetes). According to Egorov (5), effect of neomycine to many bacteria is greater. So, these results confirm similarity of this therapeutics.

Resistance, impact of antimicrobial additives, as well as monitoring of change of susceptible bacteria to chemotherapeutics have been interesting to scientist for a long period (9,12). Other studies also support our results and statements. Kelley et al. (6) tested resistance of enterobacteria and *P. aeruginosa* isolated from cover for chicken treated with 12 different antibiotics. Results of this experiment showed that strains were multi resistant to them. Bailey et al.(1)discovered that different antimicrobial additives in different combination had impact on decreasing of number of *Salmonella sp.* in chicken caecum. Tassios at al. (11) monitored decreasing in number of infections caused by *S. enteritidis* during seven years in Greek. They found that strains resistant to ampicillin and doxycyclin showed cross resistance whit therapeutics from other classes, especially sulphonamide and streptomycin. Examination of other bacteria also showed that low doses of antibiotics have impact on resistance occurrence. For example, after usage of subinihibitory doses of linkocyn for treatment diseases caused by *Staphilococcus aureus* in cow, isolates from milk show resistance and change in morphology (8).

4. CONCLUSION

According to our results, it is obvious that resistance problem is ecological. In the competition between resistant and sensitive bacteria, antibiotics act as an ecological factor that encouraged growth of resistant strains. Also, antibiotics have a mutagenic effect to bacteria, because strains treated with low concentration of amoxicillin and penicillin in laboratory, show significant level of increasing the resistance to other antibiotics.

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