

Researches Concerning the Sensibility to Neomycin of Some Bacterial Strains Isolated from Chicken

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Abstract. In this study we aimed to determine neomycin effect on Gram negative bacterial strains of the species *Escherichia coli*, *Salmonella spp*, *Proteus spp* and *Klebsiella pneumoniae* isolated from chickens with different diseases. Sensitivity to neomycin was also appreciated for standard reference strains: *Bacillus anthracis* 1190 R, *Staphylococcus aureus* 6538P, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *Salmonella enteritidis* ATCC 13076. The experiment took place at the department of Microbiology of the Faculty of Veterinary Medicine Cluj-Napoca within October 2010 - March 2011, realizing the sensitivity tests to neomycin using the diffusimetric method. After testing a total of 33 bacterial strains, the resistance to neomycin was observed for 3 species (*Escherichia*, *Proteus* and *Klebsiella*) while for genus *Salmonella* no resistant strains were registered. Neomycin resistance was of 20% for strains of *Escherichia*, 25% for *Proteus* and 33.3% in those from genus *Klebsiella*. Sensitivity percentage of the tested strains ranged between 25% and 66.6%. Percentage of moderate sensitive strains ranged from 0 to 50%. Of the 6 standard reference strains tested, one was classified as sensitive (*Staphylococcus aureus* 6538P), three resistant (*Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *Salmonella enteritidis* ATCC 13076) and two moderate sensitive (*Bacillus anthracis* 1190 R and *Bacillus cereus* ATCC 14579). The results obtained reveals that resistance to neomycin for the strains isolated from chickens varied from one bacterial species to another. Infections involving *Escherichia coli*, *Proteus spp*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* should be considered more carefully, neomycin being recommended for the treatment only when the outbreak strains were tested and classified as sensitive to this antibiotic.

Keywords: neomycin, antimicrobial resistance, bacteria from chicken

INTRODUCTION

Neomycin is an aminoglycoside antibiotic, with a similar antibacterial spectrum to streptomycin. It is especially active against Gram-negative aerobic bacilli. Has a weak activity against streptococci and *Pseudomonas*. Its activity is bactericidal (Cernea, 2009).

Microbial resistance to antibiotics is installed gradually especially after repeated and prolonged treatments. This is due to exposure to antibiotic and could be plasmid mediated to some microorganisms. The emergence of resistance may occur due to active transport system deficiencies or reduction of membrane permeability, which makes the antibiotic concentrations do not perform active in bacterial cells.

Active transport that neomycin enters bacterial cells is dependent on the presence of oxygen in the environment, which explains the natural resistance of anaerobic bacteria. Inside bacterial cells, aminoglycoside binds to 30S ribosomal subunits, resulting errors in the mRNA

with the consequence of inhibition of bacterial protein biosynthesis. Bacterial resistance is the result of enzymes that inactivate aminoglycosides by adenilation, acetylation or phosphorylation, or changes in the bacterial wall.

In this study we aimed to update the data on the resistance of microbial species isolated from lesions in chickens compared to some standard reference strains to neomycin, an antibiotic used in the therapy of microbial affections.

MATERIALS ŞI METHODS

In this study we aimed to determine neomycin effect on Gram negative bacterial strains of the species *Escherichia coli*, *Salmonella spp*, *Proteus spp* and *Klebsiella pneumoniae* isolated from chickens with different diseases. Sensitivity to neomycin was also appreciated for standard reference strains: *Bacillus anthracis* 1190 R, *Staphylococcus aureus* 6538P, *Bacillus cereus* ATCC 14579, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 10536 and *Salmonella enteritidis* ATCC 13076. A total of 33 different strains isolated from disease outbreaks in domestic birds (chickens) and 6 strains of collection were considered within the study. The experiment took place at the department of Microbiology of the Faculty of Veterinary Medicine Cluj-Napoca within October 2010 - March 2011, achieving identification test microorganisms by classical bacteriological methods (microscopic exam, cultural and biochemical tests), serological tests and automatically identification using API bioMérieux system. Isolates were tested for sensitivity to neomycin by the diffusimetric method.

RESULTS AND DISCUSSIONS

Tests of microorganism's resistance to treatment with neomycin showed that the phenomenon of resistance of microorganisms to neomycin had a slow evolution for microbial agents involved in the production of primary and secondary infection in chickens produced by bacteria sensitive to this antibiotic.

In support of these statements are representative the results presented in Table 1 and 2 and in Chart 1, where data are obtained from tests of resistance to neomycin, of some bacterial strains isolated from chickens and standard reference strains from American Type Culture Collection (ATCC).

The results situation for neomycin must be interpreted considering Gram negative bacteria possibility of adapting to antibiotics. Only for *Salmonella spp*. was observed sensitivity to neomycin, other 3 species (*Escherichia coli*, *Proteus spp*. and *Klebsiella pneumoniae*) resistant, but with low rates of resistance, between 0 and 33.33% (chart 2).

Laboratory tests with neomycin leded to resistance between 0 and 33.33% of the strains tested. The sensitivity obtained was between 25 and 66%. For this antibiotic resistant strain of *E. coli* was of only 20% (Table 1).

Resistance to neomycin was tested in laboratory conditions for 6 bacterial strains of different species, standard reference strains of American Type Culture Collection. Laboratory results obtained and processed according to CLSI standards - 2009 revealed that strains of *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 10536, *Salmonella enteritidis* ATCC 13076 were resistant to neomycin while strain of *Staphylococcus aureus* 6538P was sensitive to this antibiotic. Strains of *Bacillus anthracis* 1190 R and *Bacillus cereus* ATCC 14579 were classified as moderate sensitive.

Table 1.

Neomycin resistance situation of bacterial strains tested

Strains tested	Resistant (nr.)	Moderate sensitive (nr.)	Sensitive (nr.)	Total strains (nr.)	Resistant (%)	Moderate sensitive (%)	Sensitive (%)
<i>Escherichia coli</i>	4	6	10	20	20	30	50
<i>Salmonella spp.</i>	0	2	4	6	0	33.33	66.66
<i>Proteus spp.</i>	1	2	1	4	25	50	25
<i>Klebsiella pneumoniae</i>	1	0	2	3	33.33	0	66.66

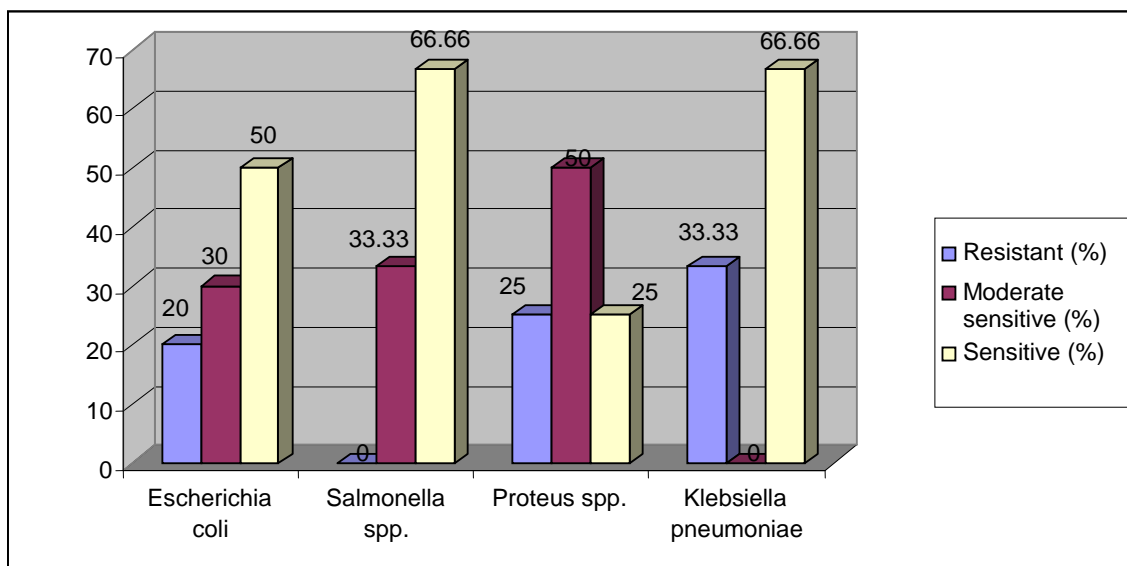


Chart 1. Graphical representation of resistance to neomycin of some bacterial strains

Table 2.

Resistance to neomycin of some strains of American Type Culture Collection (ATCC)

Antibiotic tested	American Type Culture Collection strains tested					
	<i>Staphylococcus aureus</i> 6538P	<i>Bacillus anthracis</i> 1190	<i>Bacillus cereus</i> ATCC 14579	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Escherichia coli</i> ATCC 10536	<i>Salmonella enteritidis</i> ATCC 13076
Neomycin 30µg	18 (S)	15 (M)	16 (M)	0 (R)	11 (R)	10 (R)

S-sensitive; M-moderate sensitive; R-resistant;

Making an overall assessment of the neomycin resistance is observed that from 8 species tested, for two of them no resistant strains were found (*Staphylococcus aureus* 6538P and *Bacillus spp.* strains tested) while for 5 (*Escherichia coli*, *Proteus spp.*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Klebsiella pneumoniae*) was registered resistance. Resistance values were between 0 and 33.3% and the sensitivity to this antibiotic was between 25 and 66% (Chart 1).

Making a comparison of data obtained by us on resistance to neomycin of pathogens isolated from animals with other data obtained by other researchers in the field on pathogens isolated from chickens in different areas, we consider the situation of resistance levels as satisfactory in the our tests. In this context we mention some results obtained by some researchers who surveyed resistance strains in chickens at the country level. In a large study of neomycin resistance of bacteria of avian origin, Tudor (2008) noted that the testing of 448 strains of *E. coli* obtained a sensitivity of 80% of strains tested while 15% were resistant. On staphylococci the same author obtained a sensitivity rate of 76%, while 14% of the trains were considered resistant. Resistance situation for *Pseudomonas aeruginosa* strains was different from results obtained by the same author, only 4% of strains were sensitive and 56% were considered resistant.

Similar but more encouraging data were recorded for these bacterial species where a rate of 20% of strains tested to be resistant to neomycin was observed.

It seems that resistance to antibiotics is influenced by farming systems, in this regard is found that neomycin resistance is higher in *E. coli* strains isolated from intensive chicken farms where a resistance of 88% was registered, compared to strains isolated households where he observed a 90% of strains resistant to neomycin (Shtylla Tana et al., 2009).

Sensitivity differences to neomycin between bacterial species can be determined using minimum inhibitory concentration (MIC). Thus the minimum inhibitory concentrations obtained fir neomycin on bacterial strains isolated from animals are different depending on bacterial species, so for *Staphylococcus aureus* was 0.5 µg/ml, for *Escherichia coli* is 0.8 µg/ml, for *Klebsiella pneumoniae* to 0.2 µg/ml while for *Proteus mirabilis* 0.8 µg/ml (Moellering, 1983).

CONCLUSIONS

After analyzing the results of this study and literature data, is observed that that resistance to neomycin is more exacerbated in most strains studied by us and that it varies from one bacterial species to another according to geographical area studied. However it can be stated that infections involving microbial species which frequently acquire resistance, especially *E. coli* and *P. aeruginosa*, this antibiotic may be recommended only after prior resistance testing.

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