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# ASPECTS OF IN VITRO SYNERGISM, REGISTERED IN ANIMALS

Chereji Anca\*, Chereji R.\*\*, Cernea M.\*, Oros A.\*

\*University of Agricultural Sciences and Veterinary Medicine Cluj, Faculty of Veterinary Medicine, 3-5 Manastur St., Cluj-Napoca, Romania \*\*S.C. Biovet Serv. S.R.L., Primaverii f.n. St., Cluj-Napoca, Romania

#### Abstract

Disk diffusion method was used to test the sensitivity of pathogens isolated from various biological samples, taken from ruminants, pigs and dogs. The interpretation of antibiograms revealed potentiation and synergism phenomena between antibiotics. Synergism was found between amoxicillin and streptomycin and also was registered the fact that amoxicillin enhances the activity of erythromycin in staphylococci. Could be observed synergism effect between colistin and flumequine in a strain of Neisseria spp. We noticed potentiating effect induced by amoxicillin and amoxicillin/clavulanic acid on florfenicol, in a strain of the Streptococcus, and also the presence of synergism in strain of Escherichia coli between florfenicol and amoxicillin/clavulanic acid. So, the increased antibacterial effect urges the recommendation to use combinations of antibiotics in therapy.

Key words: antibiotics, synergism, animals

#### INTRODUCTION

Antibiotics are used to treat and control many types of infections in a variety of animal species. Bacterial resistance can occur as a consequence of antibiotic use in animals and humans.

Development of resistance can be reduced by measures designed to extend the use of antibiotics. One of these measures is the use of the drug combination therapy (Mărculescu Anca, 2007).

The purpose of this work is to increase attention on the issue of antibiotic resistance and to recommend antibiotic combinations that prove to be synergistic, for better therapeutic efficacy.

## MATERIAL AND METHOD

*Pathological material.* Consisted of milk, uterine secretions and scrapes of the skin, which were taken from ruminants, pigs and dogs from households and also from various farms, including the farm of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

*Culture media.* Were used broth, agar and enteric agar CMV medium, to which were added as appropriate, glucose, serum or blood (Răpuntean Gh. et al, 2001).

Antimicrobial substances. Antibiotic standardized discs were used in antibiograms, being available from various companies. The small discs contain antibiotics from the groups of penicillins, cephalosporins, aminoglycosides, quinolones, macrolides, lincosamides, polymyxins, tetracyclines, phenicols and sulfonamides.

*Bacterial strains*. The strains isolates in this study belonged to the genera *Staphylococcus, Streptococcus, Neisseria, Escherichia*.

*Method.* The bacterial strains were tested for sensitivity to antibiotics with disk difussion method and the interpretation has been achieved according to international standards (NCCLS M31-A, M31-T, 1999).

### **RESULTS AND DISSCUSIONS**

Aspects of synergism between antibiotics have been observed, as follows:

#### Strain isolated from mastitic milk

In a dairy farm there were frequently reported mastitis. A milk sample has been received from one of the sick cows in order to do the laboratory examination.

Bacterioscopic examination found gram-positive cocci, which were identified after bacteriological examination as being staphylococci.

Isolated strain was tested against some antibiotics: amoxicillin, streptomycin, gentamicin, oxytetracycline, ceftiofur, erythromycin and penicillin (table 1).

Table 1

Antibiogram results for a Staphylococcus strain isolated from a cow with mastitis

	Antibiotic – inhibition area (mm)							
Strain	Amoxicillin	Streptomycin	Gentamycin	Oxytetracycline	Ceftiofur	Eritromycin	Penicillin	
Staphylococcus	22*•	R•	20*	22*	22	28	R	

\* resistant colonies in the inhibition area

synergism between amoxicillin and streptomycin

The strain showed susceptibility to ceftiofur and erythromycin, and resistance to penicillin and streptomycin. In the inhibition area of amoxicillin, gentamicin and oxytetracycline have developed many resistant colonies, interpreting the pathogen as being resistant to these antibiotics. We mention that a synergy effect appeared between amoxicillin and streptomycin. The treatment based on the combination of these antibiotics resulted in the healing of animals.

Combinations of aminoglycosides and other antibiotics may produce also synergism, as Enteza mentioned in 2009: tigecycline with amikacin showed synergy for 40-100% of *Enterobacter spp., Klebsiella pneumoniae, Proteus spp.* and *Stenotrophomonas maltophilia* isolates; moreover, bactericidal synergism occurred with tigecycline plus amikacin against problematic *Acinetobacter baumannii* and *Proteus vulgaris*, and with colistin against *K. pneumoniae* (Enteza JM et al, 2009).

### Strain isolated from a cow with mastitis

A sample of milk from a cow with mastitis, rebellious to treatment, was received for bacteriological and bacterioscopic examination. It has been identified and isolated a strain of *Staphylococcus* that was tested against antibiotics.

It was noted the occurrence of a potentiation effect between amoxicillin and erythromycin. The amoxicillin potentiate the action of erythromycin which extends its inhibition zone toward this antibiotic, more precisely in the area with resistant colonies around the disc containing amoxicillin (table 2).

Table 2

	Antibiotic – inhibition area (mm)							
Strain	Kanamycin	Streptomycin	Neomycin	Amoxicillin	Erythromycin	Tetracycline	Penicillin	
Staphylococcus	R	R	20	R•	18•	12	R	

Antibiogram results for a Staphylococcus strain isolated from a cow with mastitis

• potentiation between amoxicillin and eyithromycin

Studies carried out by polish scientists demonstrate the synergistic effect of cefixime and amoxicillin combination (Grzybowska W, 2004) while other researchers (Aslangul E. et al., 2005) observed either no

bactericidal effect of the combination or synergism or that the combination therapy with amoxicillin and gentamicin was significantly more active than amoxicillin alone, on different strains of *Enterococcus faecalis*.

Synergy with vancomycin was also found by modified disk diffusion testing for ceftriaxone, ceftazidime, cefpodoxime, and amoxicillinclavulanate for some strains of stapylococci (Climo MW et al., 1999).

## Strain of Neisseria isolated from dog

A strain of *Neisseria spp.* was isolated from a German Shepherd dog, aged 1 year, having interdigital ulcerative lesions, with a tendency to expand in neighboring areas.

The result of antibiogram is shown in the following table (table 3) :

Table 3

	Antibiotic – inhibition area (mm)								
Strain	Oxytetracycline	Trimethoprim/ Sulfamethoxazole	Amoxicillin	Gentamycin	Colistin	Flumequine	Penicillin	Clindamycin	Neomycin
Neisseria spp.	R	R	12*	20	10*•	26*•	R	R	12

Antibiogram results for a *Neisseria* strain isolated from dog

\* resistant colonies in the inhibition area

• synergism between colistin and flumequine

You can find that the isolated strain has a multiple resistance, being sensitive only to gentamicin. Can be noted a synergy effect between colistin and flumequine, showed by the existence of an area of lenticular aspect between the two antibiotics, where no colonies developed.

A synergistic effect was detected also by a group of reserchers from National Institute for Infectious Diseases L Spallanzani, Rome, Italy, by examining the combination of colistin and rifampicin for Pseudomonas aeruginosa (Petrosillo N. et al, 2008).

In combination, maximum synergy was observed with colistin plus rifampicin, for a multi-drug resistant Pseudomonas aeruginosa, by japanese scientists in Tokyo (Aoki N. et al, 2009).

# Strains isolated from sows with endometritis

In a pig breeding farm, sows were registered, after calving, with various forms of endometritis, resistant to treatments performed.

Bacterioscopic examination showed a diverse bacterial flora consisting of gram-negative pathogens with typical morphology for enterobacteria and also pathogens from gram-positive cocci group.

Sensitivity test was carried out and the results of antibiogram are presented in the table below (table 4):

Table 4

	Antibiotic – inhibition area (mm)					
Strain	Florfenicol	Amoxicillin	Amoxicillin/ clavulanic acid			
Streptococcus (1)	44	26*	24*			
Streptococcus (2)	34	28	24			
Escherichia coli	46*	R	R			

Antibiogram results for strains isolated from sows with endometritis

\* resistant colonies in the inhibition area

It can be seen that the largest inhibition zones were observed in florfenicol and were appreciated as sensitivity of the bacterial strains to this antibiotic.

We registered the potentiating effect induced by amoxicillin and amoxicillin/clavulanic acid on florfenicol, in the strain no. 1 of the *Streptococcus* genus. Synergism was observed also in the strain of *Escherichia coli*, between florfenicol and amoxicillin/clavulanic acid.

As a possible solution to the problem of resistance of streptococci may be the effectiveness of a combination of cefixime and amoxicillin which have a stronger activity against these microorganisms in comparison with a monotherapy, as was observed by a researcher from Osaka (Matsumoto Y., 1998).

The occurrence of synergism and potentiation phenomena between antibiotics, with enhances antibacterial effect, urges to the recommendation of the combinations of antibiotics in therapy based on antibiogram results.

#### CONCLUSIONS

- The synergism phenomenon between amoxicillin and streptomycin was found in a strain of *Staphylococcus* isolated in a sample of milk from a farm of cows with mastitis
- ✤ We mention also the appearence of a potentiating effect between amoxicillin and erythromycin in a strain of *Staphylococcus* isolated from a cow with mastitis rebellious to treatment
- Synergism between colistin and flumequine could be observed in case of *Neisseria* strain isolated from a German Shepherd with interdigital lesions unlcerative
- Potentiating effect induced by amoxicillin and amoxicillin/ clavulanic acid on florfenicol was registered in a strain of the *Streptococcus* genus, and synergism between florfenicol and amoxicillin/clavulanic acid in *Escherichia coli*, these strains being isolated from sows with endometritis
- Therefore, the occurrence of synergism and potentiation phenomena recommend the combinations of antibiotics in veterinary therapy

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