

DETERMINATION OF PATHOGEN RESISTANCE TO STREPTOMYCIN

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Abstract

This research was made in order to emphasize the actual incidence of the sensibility of various bacterial pathogens to streptomycin. The pathogens were identified as belonging to *Staphylococcus*, *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Proteus*, *Pasteurella* and *Pseudomonas* genus.

Antimicrobial agar disk diffusion method was used to test the strains isolated from animals and the results were interpreted as resistant, intermediate and sensitive according to international standards.

Resistance in 100% percentage was registered for staphylococci and also for the pathogens from *Citrobacter*, *Enterobacter*, *Proteus*, *Pasteurella*, *Pseudomonas* genus. There was also resistance for *Escherichia coli* strains - 90%, and *Salmonella* - 50%.

Intermediary values of the antibacterial inhibition zones presented *Salmonella* (50%) and also *Escherichia coli* pathogens (10%).

The only genus that was sensitive to streptomycin was *Klebsiella*.

The frequency of resistance phenomenon to streptomycin was 87,5%, intermediary values for the inhibition zones – 8,33% and only 4,17% of the strains showed sensibility to this antibiotic.

Keywords: pathogen resistance, streptomycin

Introduction

Aminoglycosides represent, next to betalactamines, the group of antibiotics having the larger therapeutical use. The first discovered aminoglycosides were natural molecules, produced by *Streptomyces* (streptomycin, neomycin, kanamycin, tobramycin) or *Micromonospora* (gentamycin, sisomicin).

Streptomycin and dihydrostreptomycin have a relatively restricted spectrum, the resistance being very spread between these antibiotics. Nevertheless, there are a few staphylococci, even some streptococci and gram-negative bacilli still sensitive, among these *Actinomyces bovis* strains, *Pasteurella spp.*, *E. coli*, *Salmonella spp.*, *Campylobacter fetus*, *Leptospira spp.* and *Brucella spp.* *Mycobacterium tuberculosis*, also, is still sensitive to streptomycin (Vakurenko S.B. and Mobashery S., 2003).

Starting with natural aminoglycosides, there were later conceived, using semisynthesis technique, molecules less sensitive to enzyme inactivation induced by resistant bacteria and having lower toxicity (amikacin, netilmicin etc.) (Oniga O. and Tiperciuc Brânduşa, 2003).

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This study was made in order to investigate the present resistance of some pathogens to streptomycin, taking into account the fact that aminoglycosides are used, despite their high nephrotoxicity, to control local and systemic infections due to sensitive aerobic bacteria (generally gram-negative) (Cristina T. Romeo, 2006). The aminoglycosides recommendation is justified by their therapeutical efficiency (Mărculescu Anca, 2007).

Material and Methods

The sensibility of a pathogen to antibiotics can be tested through a relative unsophisticated method – the antibiogram.

The materials used in this study were represented by bacterial strains, culture media and disks with a standard quantity of antibiotic.

The bacterial strains were isolated from abscesses, phlegmons, vaginal secretions, secretions mastitis, faeces, intestinal contents, organs with lesions (liver, spleen, heart, lungs, nodules etc.), bones and corpses of animals.

The susceptibility to streptomycin was tested on 48 gram-positive and gram-negative bacterial strains.

The cultivation was made on usual media (broth and agar) with a plus of serum, glucose, blood when necessary, or special media (Răpunțean Gh. and Boldizsar E., 2001).

The interpretation of the antimicrobial agar disk diffusion method had been effected according to international standards (NCCLS M31-A, M31-T, 1999).

Results and Discussions

A number of 48 pathogens from *Staphylococcus*, *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Proteus*, *Pasteurella*, *Pseudomonas* genus was investigated through antibiogram in order to determine the sensitivity to streptomycin.

The results were interpreted (table 1) and the pathogens were noted as resistant (R), sensible (S) or with intermediary values (I) to this antibiotic (table 2).

Table 1. Disc diffusion test interpretation according to international standards

Crt. no.	Antibiotic	Atb/conc. disc	Diametrul zonei de inhibiție antibacteriană (mm)			Standard values:
			R	I	S	
1	Streptomicină	100 µg	≤ 22	23 – 25	≥ 26	Neo-Sensitab Veterinary Pathogens –NCCLS
	Streptomicină	10 µg	≤ 11	12 – 14	≥ 15	BBL Sensi-Disc – NCCLS

Table 2. Susceptibility/resistance to streptomycin

Crt. no.	Bacterial strains	No.	Sensibility/resistance					
			R	%	I	%	S	%
1	<i>Staphylococcus</i>	6	6	100	-	-	-	-
2	<i>E. coli</i>	20	18	90	2	10	-	-
3	<i>Citrobacter</i>	2	2	100	-	-	-	-
4	<i>Enterobacter</i>	2	2	100	-	-	-	-
5	<i>Klebsiella</i>	2	-	-	-	-	2	100
6	<i>Salmonella</i>	4	2	50	2	50	-	-
7	<i>Proteus</i>	4	4	100	-	-	-	-
8	<i>Pasteurella</i>	2	2	100	-	-	-	-
9	<i>Pseudomonas</i>	6	6	100	-	-	-	-
Total no. of pathogens		48	42	87,50	4	8,33	2	4,17

Many attainment mechanisms of resistance to streptomycin were described; can be plasmid mediated or owed to mutations. A non-plasmid mediated mechanism is the decrease of the transportation through cellular membrane. The anaerobic (*Clostridium*) and facultative anaerobic (enterobacteriaceae and some staphylococci) bacteria are more resistant to aminoglycosides, in an anaerobic medium, because of the active and oxygen-dependent transportation.

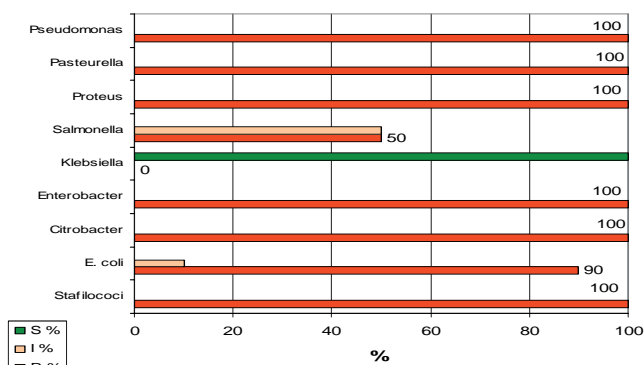
A 100% resistance of staphylococci can be observed in the antibiograms of the present study. The resistance due to decreased carriage can be induced by the exposal of bacterial strains to sublethal concentrations of antibiotic. An example is the *Pseudomonas aeruginosa* resistance to streptomycin, as it is obvious also in the tested strains of this research.

The decreasing of the binding of antibiotic to ribosomes is also a mechanism of *Pseudomonas* resistance to streptomycin; that type of mechanism is met also in *Escherichia coli* strains. Furthermore, the results of the present study show a 100% percentage of resistance in *Pseudomonas*, whilst in the case of *Escherichia coli* – a 90% resistance and 10% intermediary values of the antibacterial inhibition zones; as some authors point out (Angelescu M., 1998), that means also 100% resistance, because mostly statistical and epidemiological studies present the existence only of resistant and sensible strains.

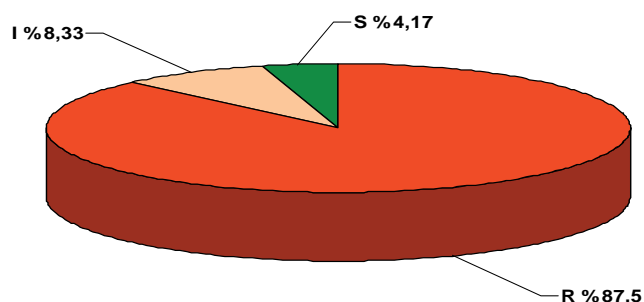
Even if Ray A. et al, in 2006, observe in *Salmonella* genus the highest resistance to streptomycin, in this research there are only 50% of resistant strains; all the same, because of the existence of a great number of intermediate strains – 50%, the sensitivity of the pathogens to this antibiotic is 0%.

The resistance emerge also through an enzymatic mechanism. These enzymes are found both in gram-negative and gram-positive bacteria. It is possible that this mechanism determined the resistance in all strains from *Citrobacter*, *Enterobacter*, *Proteus* și *Pasteurella* genus, as it is showed in graphic 1.

Accordingly, it is observed that for streptomycin the antibiotic resistance phenomenon appear in a rather high percentage – 87,5%, as we marked for 21 of the 24 tested strains, being in existence also a percentage of 8,33% of intermediary values – 2 strains, and only one strain was sensitive – a percentage of 4,17% of the total strains (graphic 2).



Graphic 1. Resistance to streptomycin



Graphic 2. The frequentness of resistance phenomenon to streptomycin

Conclusions

- Bacterial strains from *Staphylococcus*, *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Proteus*, *Pasteurella*, *Pseudomonas* genus were investigated about the susceptibility to streptomycin;
- The antimicrobial agar disk diffusion method was used for the testing of the pathogens;
- The results were interpreted according to international standards;
- Staphylococci registered a 100% resistance to streptomycin and also *Citrobacter*, *Enterobacter*, *Proteus*, *Pasteurella*, *Pseudomonas* strains;

- *Escherichia coli* strains showed also rather high percentage of resistance to this antibiotic – 90%, the rest of 10% being intermediary values of the inhibition zones
- Resistance of 50% was observed for *Salmonella* pathogens and intermediary values, also in 50% percentage
- The only genus that was sensitive to streptomycin was *Klebsiella*;
- The resistance of the investigated pathogens was, generally, 87,5%, being noted intermediary values – 8,33% and only 4,17% of the strains showed sensibility to this antibiotic.

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