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ANTIBIORESISTANCE OF BACTERIA – INCREASING CHALLENGE

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ABSTRACT

A total of 145 samples from bovine, bird, pigs, dogs, slaughterhouse and people were tested for antibioresistance during the period 2003-2004. A total of 281 isolates were studied. 62 of them were determined as E.coli, 65 as Staphylococcus aureus, 54 as Enterococcus faecium and Enterococcus faecalis The in vitro susceptibility of bacterial substances was tested by agar diffusion method on Muller-Hinton agar. The results were defined by measuring the diameter of the zones of inhibition and for interpretation the three-stage system of Kirby-Bauer was used. The data indicated that there is an increased resistance by isolated bacteria to the common antibiotics in use in the animal farms. Furthermore, there was a conformity of antibioresistance in organisms, isolated from animals and from personnel, caring for or being in contact with them (farmers, calf breeders, cow breeders, pig breeders, slaughterhouse workers, dog owners etc.). An exchange of antibioresistant microflora between animals and men or only a transfer of genetic material (integrons), coding this resistance with high phenomenon prevalence was most probably occurring - a fact with significant epidemiological and medical importance for practice. This danger could be reduced by immunization of animals and people, adequate disinfection and deratization of premises, contaminated with resistant microorganisms, periodical screening and monitoring for detection of resistant bacteria in animal farms and human hospitals. The indications, terms and doses of antibiotic use are to be followed very strictly

Using antibiotics to control and treat diseases in animals improves the safety of our food supply by providing healthier sources of meat, cheese, milk and eggs. Meanwhile, in the USA, it is estimated that some 60-80% of all cattle, sheep, swine and poultry receive some 9500 tons of therapeutic or subtherapeutic antibiotics annually.(Gill , 2003;.).But there have been a few cases in which bacteria that resist antibiotics can be passed from food animals to humans and vice-versa creating a public health risks - microbe-related illnesses in humans - meningitis, endocarditis, arthritis, pneumonia, bronchitis, outbreaks of enzootic of coliinfections, infections with streptoccocus and staphyloccocus spp. For instance, in 2004, two cases of antibioresistant bacterial infections are reported in Bulgaria: one caused by an Erisypelotrix bacteria and subsequent endocarditis and arthritis in a veterinarian and the other, caused by a meningococcus, resulting in a lethal infection of a student in a secondary school of veterinary medicine. Extra vigilance is needed to prevent the spread of bacteria that cannot be destroyed with currently available drugs. In Denmark, the use of antibiotics per kilogramme of weight gain was on the average 43 mg/kg in swine, 96 mg/kg in fish, 135 mg/kg in working people, 52 mg/kg in cattle, 0,5 mg/kg in birds. New Zealand and Denmark have banned antibiotics for growth promotion in food animals, as the European Union.. The impending ban on AGPs (antibiotic growth promoters) will probably increase the sensitivity to diseases. The feed grade AGPs will be banned largely based on the view that cross-resistance of human pathogens to antibiotics used in human medicine would be reduced.

Maintaining animal health without AGPs will remain an important topic. Also, further increases in animal performance, based on genetic advances, could make animals more sensitive to disease.(Gill and .Best ., 1998 .; Gill , 2003)

Together with its unquestionable properties, the use of antibiotics is also accompanied by several problems related to adverse effects such as allergenicity, immunosuppression, toxicity and the ensuing systemic homeostasis alterations. The most important problem however, is the role of antibiotics as a factor in the selection of bacterial strains, resistant to one or more antibiotics, i.e. the problem of antiobioresistance.

The antibioresistance of bacteria is a big challenge to the successful therapy of infectious diseases in both humans and animals. On one part, the problem is with the weak therapeutical effect and on the other, in the possibility of dissemination and conservation of resistant strains in the ecosystem, that, under favourable conditions, could provoke epidemic (nosocomial/hospital acquired infections) and epizootic (pens-specific infection) infectious outbreaks.

The antibioresistance of bacteria, as a phenomenon of the ecosystem, could be stationary (i.e. to persist with time and to be transmitted via a direct contact among men and animals). In this connection, the hospitals and the animal farms are ecosystems with an increased selection weight because of the extensive application of antibiotics and the concentrations of organisms with reduced defense mechanisms, i.e. those ecosystems are sites of selection, traffic and dissemination of antibioresistant bacterial strains.

In order to diminish the risk of appearance of antibioresistance in bacteria, several steps are undertaken, such as control upon the prescription and the use of antibiotics in animal husbandry, human and veterinary medical practice, improvement of hygiene, increasing the general and the specific resistance in men and animals, epidemiological survey and measures for decreasing the cross-infection between carriers of resistant strains and healthy individuals or groups of animals or humans.

The goal of this investigation is to monitor the trends in antibiotic resistance of some bacteria and its prevalence among food animals and people.

MATERIALS AND METHODS

Sampels. A total of 145 samples were tested during the period 2003-2004. 35 of them being from bovine farm, 70 of them being from poultry farm and slaughterhouse, 20 from pig's farm, 20 from dogs and their owners.

Isolates. A total of 281 isolates were studied. 62 of them were determined as E.coli, 65 as Staphylococcus aureus, 54 as Enterococcus faecium and Enterococcus faecalis. The microorganisms were isolated from feces and skin-wash of workers serving and contacting with the animals, from feces, skin-wash, uterus and udder secret materials of the animals, from transportation and technological machinaries. Isolation, identification and typing of the isolates was carried out according Bergey's manuals(1986).

Antibiograms. The in vitro susceptibility of bacterial substances was tested by agar diffusion method on Muller-Hinton agar. For this purpose discs with the following concentration of antibacterial substances (μg/disc) were used: ampicillin(AmP)-25; amoxicillin(AxC)-25; carbenicillin(CrB)-100; streptomycin(S)-10; gentamycin(GN)-10; kanamycin(K)-10; chloramphenicol(CL)-30; tetracyclin(TE)-30, erythromycin(E)-15; nalidix acid(NA)-30;(NCIPD); flumequin(FL)-30(Sanoffi); enrofloxacin(EnF)-5(Bayer); oxolonic acid(OA)-30 (Virbac). The results were defined by measuring the diameter of the zones of inhibition and for interpretation the three-stage system of Kirby-Bauer was used. The zones of inhibition in millimeters were regarded as resistant as follow: ampicillin≤13mm; amoxicillin≤14mm; carbenicillin ≤19mm; streptomycin≤11mm; gentamycin ≤12mm; kanamycin ≤13mm; spectinomycin ≤18mm; tetracyclin ≤14mm; chloramphenicol ≤12mm; flumequin ≤20mm; enrofloxacin ≤17mm; norfloxacin ≤12mm..

RESULTS AND DISCUSION

The resistant pattern of the bacterial isolates from all the specimens cultured is shown in table 1, 2 and 3. The data indicated that there is an increased resistance by isolated bacteria to the common antibiotics in use in these farms. In them, the antibiotics are used for treatment of endometritis and mastitis in cows, gastroenteritis in calves and pigs, dermatitis in dogs and cats, prophylactic treatment of colibacteriosis and mycoplasmosis in poultry etc. Thus, the selection of resistant strains and reinfection of animals from the environment is happening.

Table 1- *Resistance pattern of E.coli, Enterococcus spp., and Staphylococcus aureus isolated from bovine and people*.

Source and numbers	Percent of strains resistant to					
Of isolates	GN	E	AxC	TE	CL	PEF
1.E.coli						
bovine, n=14	50	50	100	50	50	50
people, n=12	0	50	50	50	0	0
2.Enteroc.spp.						
bovine, n=10	0	0	100	0	0	0
people, n=14	0	25	25	100	0	25
3.Staph.aureus						
bovine, n=12	0	50	0	100	50	0
people, n=12	0	50	50	50	50	100

Table 2- *Resistance pattern of E.coli, Enterococcus spp., and Staphylococcus aureus isolated from chickens and people.*

Source and numbers	Percent of strains resistant to					
Of isolates	GN	E	AxC	TE	CL	PEF
1.E.coli						
chickens, n=13	0	100	66,6	100	66,6	66,6
people, n=14	0	50	100	100	50	50
2.Enteroc.spp.						
chickens, n=10	0	0	0	100	0	0
people, n=10	0	0	0	100	0	0
3.Staph.aureus						
chickens, n=12	0	0	50	0	0	0
people, n=11	0	33,3	33,3	33,3	0	33,3

Table 3- *Resistance pattern of E.coli, and Staphylococcus aureus isolated from pigs,dogs and people.*

Source and numbers	Percent of strains resistant to					
Of isolates	GN	E	AxC	TE	CL	PEF
1.E.coli						
pigs,n=10	0	0	100	100	100	0
people,n=10	0	0	0	0	0	0
2.Staph.aureus						
dogs, n=10	15	12	10	17	0	5,6
people, n=10	8	5	30	30	10	12

The results show that the highest percentage of resistance was that to tetracycline, followed by amoxycillin and erythromycin. A relatively lower resistance was observed to chloramphenicol and quinolones. Furthermore, there was a conformity of antibioresistance in organisms, isolated from animals and from personnel, caring for or being in contact with them (farmers, calf breeders, cow breeders, pig breeders, slaughterhouse workers, dog owners etc.) In those cases, an exchange of antibioresistant microflora between animals and men or only a transfer of genetic material (integrons), coding this resistance with high phenomenon prevalence was most probably occurring - a fact with significant epidemiological and medical importance for practice. It could be assumed that the ecosystem, with its biotic and abiotic factors and elements, especially

the biocenose animals-people-bacteria, is permanently reproducing, maintaining and distributing the antibiotic resistance in colibacteria, staphylococci, enterococci and other microbial species. In the other hand there are data showing that inhibition of repressor gene speads antibiotic resistance and that antibiotic use can promote the dissemination of antibiotic resistance

The obtained results showed that the use of antibiotics and their residues in animal foodstuffs (eggs, milk, meat etc.) are only one of factors for appearance and selection of resistant bacterial strains. Once developing, such a population of resistant bacteria serves as a source and pool of antibioresistance in animal sheds, farms and is transmitted to newborn, newly hatched and imported animals and birds. Moreover, a cross transmission between animals and men is observed. Thus, a disbacteriosis of such bacterial types with infectious outbreaks in animals and humans could happen and their elimination via therapy is very difficult. This thesis is supported by data of other investigators (Table 4) that reported increased antibioresistance in microorganisms isolated from hospital patients (Oronsaye and Ighedosa, 2003) and various groups of animals (Urumova, 2004). and an incraesing number of nosocomial infections(hospital acquired infections) linked with the transmission of antibiotic-resistant pathogens.(Gouget, 2004)....According Kunchev (2004) Bulgarian health's ministry find out for two years that the hospitals export internal infections; between 15 and 40% of the patiants passed through the emergency, surgery and the others health centers get some infections like pneumonia or inflammation; the main cause for these events have been the missed microbiological analysis, incorrect pescribed antibiotics and bad hygien without any desinfection.

Table 4- *Resistance pattern of different bacterial isolated*

Isolates	Total No	Percent of strains rsistant to							Authors
	Of strains	GN	E	AxC	TE	CL	S	PEF	
1.Staphyl.									
Aureus									
Dogs	112	12,5	13,4	9,8	10,7	-	-	3,6	Urumova,2004
Cow	450	18,4	25,1	22,5	27,3	-	-	3,6	Urumova,2004
People	298	40	50	100	100	83	83	33	Oronsaye and Ighedosa,2003
2.E.coli									
birds	980	20,9	--	15,4	35,8	13,6	26,2	4,5	Urumova,2004
pigs	528	24,1	12,7	19,1	23,9	15,1	32,1	5.3	Urumova,2004
people	158	56	65	100	100	100	100	47	Oronsaye and Ighedosa,20033.
3. Enterococ.									
Faecium									
Birds	480	28,4	--	10,1	--	---	47,2	--	Urumova,2004

The emerging and selected antibioresistant bacterial strains could be transmitted from animals to men and vice versa. There is evidence for the transmission via the conjugation route of multiple resistance from E.coli and Salmonella spp., initially isolated from animal faeces and then, detected in the faeces of the attending personnel. Genetic determinants of resistance could be transferred from animals to human bacterial strains of the resident intestinal microflora – there are data for increase in tetracycline-resistant intestinal bacteria isolated from farmers, in whose farms tetracycline has been included in forages for a long time. The antibioresistance is also transferred when people consume animal foodstuffs, and the animals – forages, contaminated with bacteria carrying R-plasmids, transposons or integrons containing genes of resistance. An important pool and source of antibioresistant bacteria and genes between animals and men are the fertilizers, the soil, the water and especially waste waters from farms, slaughterhouses, hospitals etc. Most genes determining multiple resistance to aminoglycosides (streptomycin, kanamycin, gentamicin) in avian E. coli organisms, are a

part of the Tn 21 transposon, that is detected in human strains as well. Quinolone-resistant (enrofloxacin, norfloxacin) E.coli strains, isolated from broiler chickens are found out together with fluoroquinolone-resistant E. coli strains isolated from faeces of healthy people in the same region. People are frequently using antibiotics (against influenza, cold, cough, diarrhoea etc.) and become also carriers and disseminators of resistance. Such people are an essential pool and source of antibioresistant microbial strains that are transmitted to other people, pets, productive and newborn animals, newly hatched birds, dairy cows, sheep and goats. There are cases of transmission of staphylococci from a man to a horse, from a man to a dog, transmission of E. coli and Salmonella spp. from men to chickens, calves etc.(Scott et all., 1988; Piriz et all., 1996). The resistance to tetracyclin means that this resistance is valid to all antibiotic of this group. The same correlation existe between the resistance to gentamycin and the other aminoglycosides.

From a clinical and epidemiological point of view, most important are transfers of multiresistant E.coli and Salmonella spp., methicillin-resistant staphylococci via the milk of lactating cows, sheep and goats or dogs and cats with staphylococcal dermatitis, vancomycin-resistant avian enterococci with poultry meat and eggs, dissemination of quinolone-resistant avian Campylobacter spp. strains between animals and men.(Haundwerger et all., 1993; Bates et all., 1993; Jones et all., 1995; Hayden et all., 1997). In Bulgaria, the percentages of tetracycline-resistant avian E. coli and Salmonella spp. were 47,1% and 37,8% respectively; the staphylococci isolated from bovine udders exhibited 32,6% resistance to tetracycline, 30.2% to erythromycin and 28.9% to lincomycin; tetracycline-resistant staphylococci isolated from dogs were 14.3% (Urumova, 2004). Gouget (2004) recorded that there is incraesing number of nosocomial infections(hospital acquired infections) linked with the transmission of antibiotic-resistant pathogens..According Kunchev (2004) Bulgarian health's ministry find out for two years that the hospitals export internal infections; between 15 and 40% of the patiants passed through the emergency, surgery and the others health centers get some infections like pneumonia or inflammation; the main cause for these events have been the missed microbiological analysis, incorrect pescribed antibiotics and bad hygien without any desinfection.

In conclusion, it must be stated that the increasing prevalence of bacterial antibiotic resistance is a serious challenge to both human and animal health. This danger could be reduced by immunization of animals and people, adequate disinfection and deratization of premises, contaminated with resistant microorganisms, periodical screening and monitoring for detection of resistant bacteria in animal farms and human hospitals. The indications, terms and doses of antibiotic use are to be followed very strictly.

REFERENCES

1. Bates J., J.Jortens, T.Griffiths (1993).Evidence for an animal origin of vancomycin-resistant enterococci. Lancet .,v.342, pp. 490-491.

2. Bergey's manual of systematic bacteriology (1986).

3. Gill C. (2003) .Feed antibiotics: Risk assessment for the 'real world'. Feed Intern., june, pp.6-9.

4. Gill C., P.Best (1998).Antibiotic resistance in USA: Scientists to look more closely.. Feed Intern., august, pp.16-17.

5. Gouget B. (2004) .Nosocomial infection control in healthcare settings: A new challenge. LabMedica Intern., v.21, #5:9-10, p.4

6. Handwerger S., B.Rancher, D.Altarae, J.Monka, S.Marchion, K.Singh, B.Murray, J.Wolff, B.Walters (1993).Nosocomial outbreak due to vancomycinq penicillin and gentamycin.Clinical infection diseases, v.16, pp.750-755.

7. Hayden M.,R.Picken, D.Sahm (1997).Heterogenous expression of glycopeptide resistance in enterococci associated with transfer of Van B. Antimicrob.agents and chemother., v.41, pp. 872-874.

8. Jonnes R.N. et all. (1995).Emerging multiply resistant enterococci among clinical isolates. Prevalence data from 97 medicine centers surveillance study in USA. Diagn.microbioly,infection diseases, v.21, pp. 85-93.

9. . Kunchev A.. (2004) The hospitals export internal infections. Twenty four hours, Sofia, #325(4760), p.7

10. Oronsaye F.E., S.U.Ighedosa (2003) .Antibiotic resistance among pathogenic bacterial isolates: Results of a study at a

Nigerian teaching hospital. LabMedica Intern., v.20, #4, 7-8, pp.10-12.
11. Piriz S., J.Valle, F.Mateus, R.Fuente, D.Cid, J.Raiz, S.Vadilli (1996). In vitro activity of 15 antimicrobial agents against resistant and methicillin susceptible staphylococcus intermedius. J.Vet.Pharmacol.Therap., v.19, pp. 118-123.

12. Scot G., R.Thomson, J.Lee, G.Ridgway . (1988) Cross-infection between animals and man: possible feline transmission of Staphylococcus aureus infection in humans? J.Hosp.Infect. v.12, pp.29-34.
13. Urumova V. (2004). Phenotype monitoring of the resistance to antibiotics of some bacteria isolated from birds, pigs, bovine and dogs in Bulgaria. Ph.D. thesis, Stara Zagora, Bulgaria.