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ANALYSIS OF ANTIMICROBIAL DRUG RESISTANCE IN ENTEROPATHOGENIC *ESCHERICHIA COLI* (EPEC) ISOLATED IN RABBITS WITH DIARRHOEIC SYNDROME

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ABSTRACT

The sensitivity of 51 enteropathogenic *Escherichia coli* (EPEC) strains, isolated from recently weaned rabbits with diarrhoeic syndrome to 13 antimicrobial drugs has been tested. The samples were obtained from six farms located in North and South Bulgaria.
The inhibition zones were interpreted by the three-degree system of Bauer- Kirby's disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) NCCLS requirements.
A phenotype analysis of resistance of isolated rabbit E. coli strains to the different groups of tested antimicrobial drugs was performed. The analysis included drawing of experimental cumulative curves and their theoretical analogues as well as determinations of the inhibition zone diameters in 50 % and 90 % of isolates, the so-called ZD_{50} and ZD_{90} correspondingly to MIC_{50} and MIC_{90}.
The resistance patterns in studied strains were determined. The highest incidence (15.6%) was that of patterns including resistance to amoxicillin, cefalotin and cefuroxime as well as the profile including resistance to beta-lactams and flu equine.

Key words: *Escherichia coli*, antimicrobial resistance, rabbits

INTRODUCTION

Intestinal infections are among the most serious health problems of industrial rabbit production. From an etiological aspect, bacteria, viruses and protozoa are involved. According to Peeters (1984) enteropathogenic E. coli bacteria together with clostridia and eimeria, are most commonly implicated in the etiology of diarrhoea in rabbits (1).
Enteropathogenic colibacteria adhere on enterocytes at specific sites and alter the cytoskeleton of the intestinal epithelium, thus impairing its functions. The lesions that provoke similar injuries are defined as A/E lesions (attaching and effacing lesions), i.e. an attachment and effacing of microvilli with consequent impairment of their functions, is occurring (2,3,4).
The antimicrobial therapy is an essential factor influencing the death rates and economical losses from one part, and from the other, the unpurposeful utilization of antimicrobial drugs results in emergence of drug resistance. The data in our country are scarce (Korudzhiiski et al.) and this necessitates a very precise consideration of both empiric therapy and the cases with multidrug resistance in rabbit *E. coli* strains (5).

MATERIAL AND METHODS

Bacterial strains. From the 72 intestinal specimens obtained from rabbits, 51 *E. coli* strains were isolated and were determined as EPEC via sero- and biotyping using methods described by Okerman and Devriese, 1985, Peeters et al., 1988.
Antibiogrammes. The sensitivity to antimicrobial drugs was determined by the disk diffusion method and interpreted using the three scale system of Bauer-Kirby, as stated by NCCLS – Performance Standards for Antimicrobial Disk Susceptibility Tests – Sixth Edition; Approved Standard, NCCLS Document M2-A6, vol. 17 № 1, 1997, and Performance Standards for Antimicrobial Disk – Susceptibility Tests Sixth Edition; Development of in vitro susceptibility testing criteria and quality control parameters for veterinary antimicrobial agent. Approved

guideline, Second edition NCCLS Document M37- A2, 2002.

The antibiogrammes were performed on Mueller-Hinton agar (National Centre of Infectious and Parasitic Diseases, Sofia) with layer thickness of 4 mm. Filter paper disks with 13 antimicrobial drugs, were used: amoxicillin, cefalotin, cefuroxime, gentamicin, chloramphenicol (produced by National Centre of Infectious and Parasitic Diseases, Sofia); neomycin, spectinomycin, doxycycline, colistin, flumequine, norfloxacin and trimethoprim/sulfonamide (Seva) and enrofloxacin (Bauer). The reference strain *E. coli* ATCC 2592 was used as control.

Cumulative curve analysis. It was performed after plotting of cumulative curves on the basis of the cumulative percentages of all inhibition zones (from the minimum one to the maximum observed) for each individual antimicrobial drug. This was done by calculation of percentages of isolates for each inhibition zone diameter. For zones without isolates, the percentages of preceding larger zones were added. Then, the cumulative percentages on the Y axis were plotted against the diameters of inhibition zones (X axis). The trendlines of experimental cumulative curves were calculated using a 6th degree polynomial approximation of experimental data. The regression equations and the correlation coefficients were also calculated by the software.

Determination of inhibition zone diameters. Similarly to the MIC range, this parameter was introduced as the difference between the largest and the smallest diameter of inhibition zones of the studied isolates.

Determination of ZD_{50} and ZD_{90}. These parameters were also defined similarly to MIC_{50} and MIC_{90} and were defined as the largest diameter where 50% or 90% of isolates are inhibited, respectively. From the point where the theoretical cumulative curve intercepts the 50% inhibition line, a perpendicular to the X-axis was drawn, and by the point of interception, the value corresponding to ZD_{50} was determined. Usually, the point was between two adjacent diameters. According to the definition, the smaller diameter was chosen because ≥50% of studied strains had inhibition zones with this or larger diameter. ZD_{90} was identically determined.

RESULTS AND DISCUSSION

The data about the sensitivity of tested *E. coli* strains to antibacterial drugs are presented on five figures.

Fig. 1. depicts the experimental and theoretical cumulative curves of amoxicillin, cefalotin and cefuroxime in rabbit EPEC isolates. The range of inhibition diameters for amoxicillin was from 6 mm to 25 mm, for cefalotin: between 6 mm and 28 mm, and for cefuroxime: between 6 mm and 21 mm. The ZD_{50} and ZD_{90} values for amoxicillin were 18 mm and 15 mm; for cefalotin: 19 mm and 16 mm, and for cefuroxime: 15 mm and 11 mm, respectively.

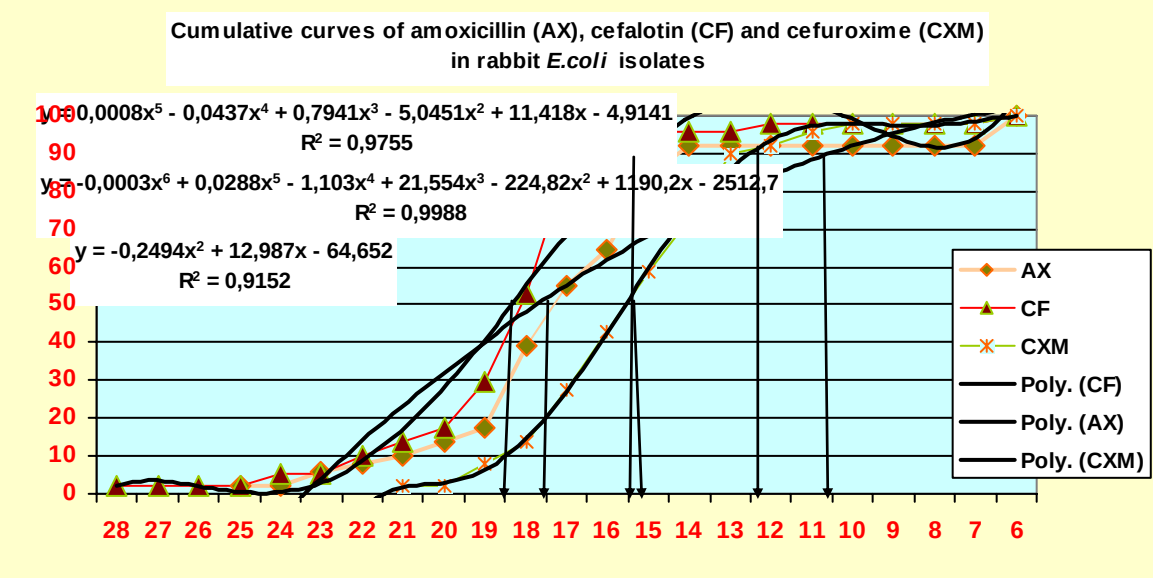

Fig. 1

Fig. 2 presents the experimental cumulative curves and the trendlines for aminoglycosides/aminocyclitols – gentamicin, neomycin, spectinomycin in septicaemic rabbit E. coli strains. The range of growth inhibition zone diameters for

gentamicin and neomycin was entirely situated in the sensitive area: from 19 mm to 38 mm for the former, and from 16 mm to 29 mm for the latter antibiotic. The inhibition zones for spectinomycin were within 17–34 mm. The respective ZD_{50} and ZD_{90} for gentamicin were 25 mm and 22 mm, for neomycin – 21 mm and 19 mm, whereas for spectinomycin – 27 mm and 19 mm.

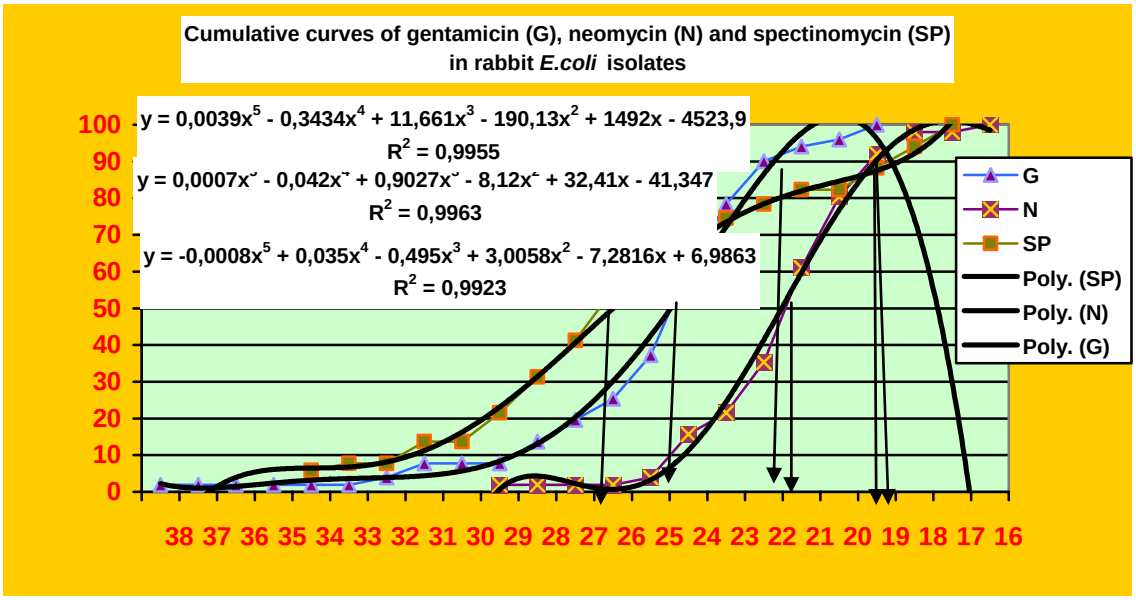

Fig.2

The experimental and theoretical cumulative curves of doxycycline, chloramphenicol and colistin in rabbit EPEC strains are presented on Fig. 3. The diameters of inhibition zones were between 8 mm and 27 mm for doxycycline, 6–31 mm fro chloramphenicol and 6-17 mm for colistin. The doxycycline ZD_{50} and ZD_{90} were 19 mm and 11 mm, these of chloramphenicol: 25 mm and 23 mm, and of colistin – 14 mm and 11 mm, respectively.

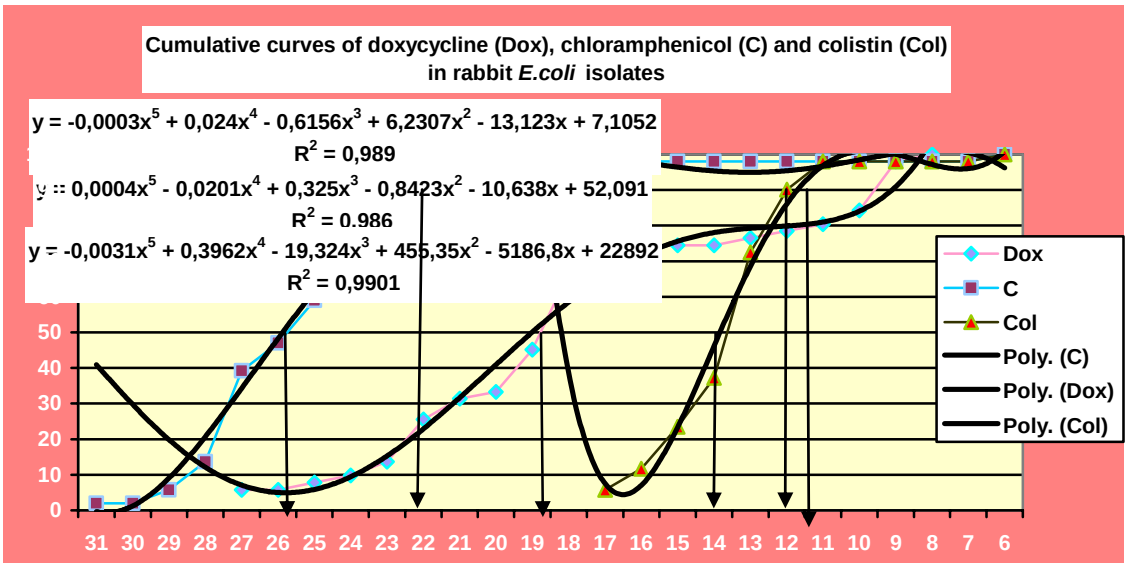

Fig.3

Fig. 4. presents the cumulative curves and respective trendlines of fluorinated quinolones: flumequine, norfloxacin and enrofloxacin in tested rabbit EPEC strains. The inhibition zone diameters were entirely in the area of sensitivity – between 22–38 mm for norfloxacin and 23–33 mm for enrofloxacin. The inhibition zone diameters against flumequine were within a broader range: from 6 mm to 32 mm. The flumequine ZD50 and ZD90 were 25 mm and 16 mm, these of norfloxacin – 31 mm and 26 mm,

and of enrofloxacin: 27 mm and 25 mm.

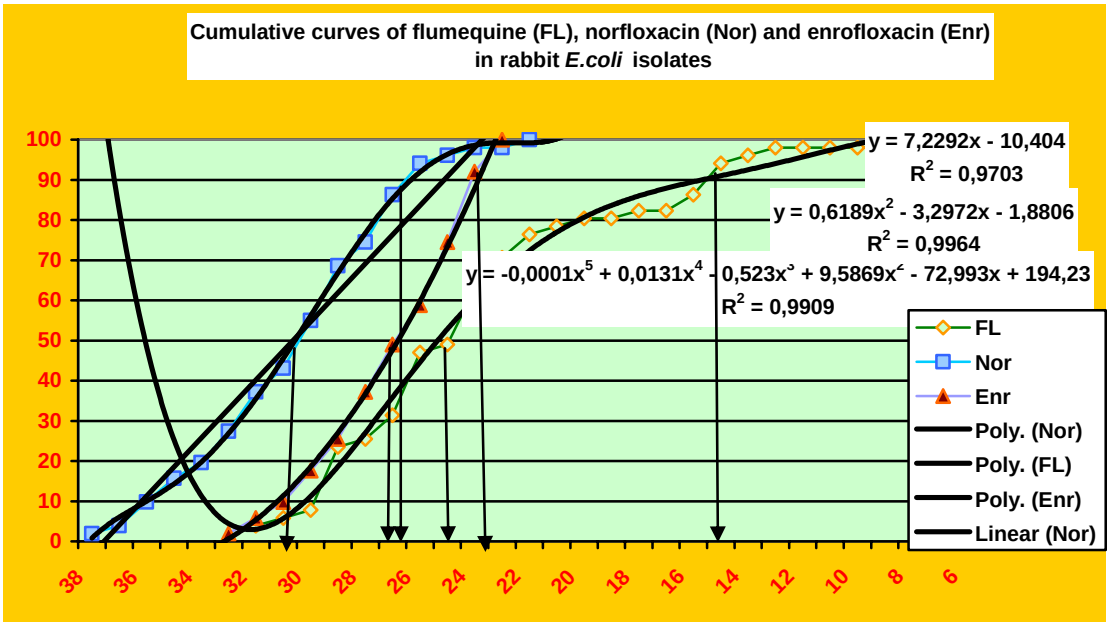

Fig.4

Fig. 5 ilustrated the cumulative curve and the trendline of the combination trimethoprim and sulfonamide in rabbit colibacterial strains. The inhibition zones were between 6 mm and 37 mm; the ZD_{50} and ZD_{90} values were 30 mm and 23 mm, respectively.

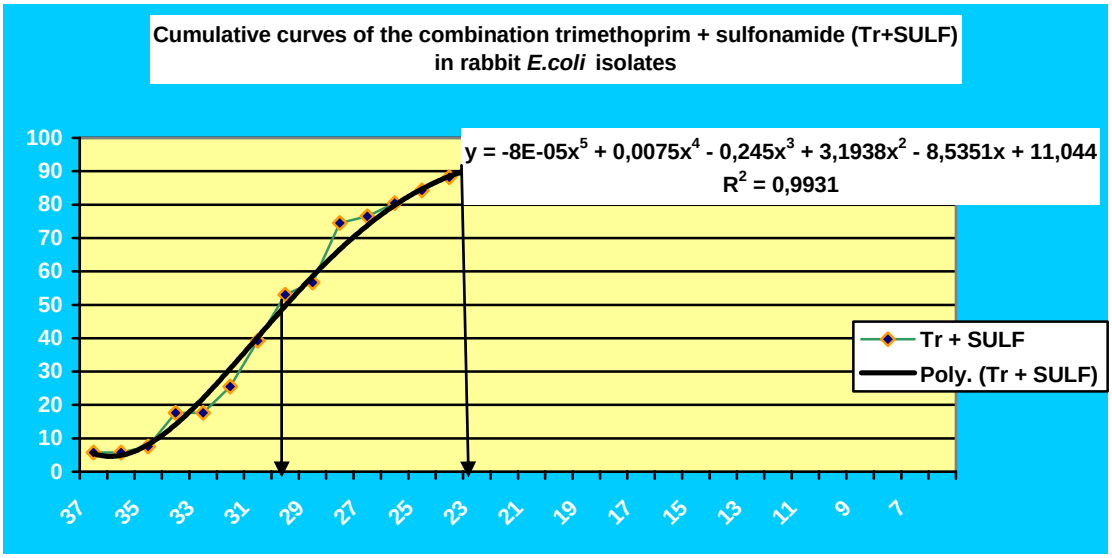

Fig. 5

The resistance patterns showed two profiles with highest percentages of 15.6 %. The first one included the resistance to three beta-lactam chemotherapeutics (cefalotin, amoxicillin and cefuroxime) and the second one included apart beta-lactams, but also flumequine. Then followed another two resistance patterns with 9.8%: the first one consisting of resistance against amoxicillin, cefuroxime and flumequine and the other, to amoxicillin and cefuroxime. The following pattern (7.8 %) included the resistance to amoxicillin, cefuroxime, doxycycline and flumequine. A profile of resistance against amoxicillin, cefalotin, cefuroxime, doxycycline and flumequine was found out in 5.8 % of isolates. Resistance to amoxicillin, cefuroxime, and doxycycline was observed in 3.9 % of tested EPEC strains.

The data evidenced a preserved sensitivity of rabbit E. coli strains to aminoglycoside-aminocyclitols: 100% against gentamicin and neomycin and 94.1% against spectinomycin.

Similar data were obtained with regard to the sensitivity of tested strains to fluorinated quinolones: the ZD_{50} and ZD_{90} values of

enrofloxacin and norfloxacin were entirely in the sensitivity zone whereas for flumequine, the range of inhibition zone diameters was broader: 6–32 mm. The ZD_{90} of flumequine was in the intermediate zone (16 mm).

The sensitivity of studied E. coli strains to chloramphenicol/thiamphenicol and colistin was preserved. Although the range of growth inhibition zone diameters was between 6 mm and 31 mm, the ZD_{50} and ZD_{90} values were in the sensitive area.

With respect to doxycycline, a broader inhibition zone range was detected: 8–22 mm, with ZD_{90} being in the intermediate zone.

The ZD50 and ZD90 for the combination of trimethoprim and sulfonamide were in the area of sensitivity.

The highest resistance percentage was determined against beta-lactam antibiotics: 90.2 % (amoxicillin), 47.06% (cefalotin), 86.2 % (cefuroxime). The ZD_{50} and ZD_{90} values for amoxicillin were in the intermediate zone; ZD_{50} of cefuroxime was in the intermediate zone but ZD_{90} – in the resistance zone – 11 mm.

Similar results were reported by Korudzhiiski and coauthors – high MIC of beta-lactam antibiotics in enteropathogenic E. coli strains in rabbits. In isolates studied by them, the resistance against cefuroxime was 100 % and that against amoxicillin: 88.2 % (5).

Camarda et al. reported a higher resistance percentage (83.9 %) against tetracycline, spectinomycin and the combination trimethoprim/sulfonamide in rabbit *E. coli* strains. Our data differ with regard to these antibiotics as we determined a high sensitivity to trimethoprim/sulfonamide as well as a higher percentage of strains sensitive to doxycycline – 76.4% (7).

According to Pisoni et al., rabbit E. coli strains had a good sensitivity against fluorinated quinolones. In our investigation, tested strains exhibited 100% sensitivity to enrofloxacin and norfloxacin (8).

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