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RESEARCH ON THE *ESCHERICHIA COLI, PROTEUS MIRABILIS, KLEBSIELLA SPP.* PHENOTYPES OF RESISTANCE TO ANTIBIOTICS

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***Key words*:** *Enterobacteriaceae, growth parameters, antibiotic sensivity,*

INTRODUCTION

Antibiotics are substances with selective action against bacteria, which have been the solution to solving bacterial infections, since their discovery and use in medical practice.

If at first the use of antibiotics to treat infections was strict and limited, now the inappropriate use and abuse of substances has led to increased resistance of bacteria to antibiotics.

Also, the use of antibiotics for preventive purposes in areas related to human food (zootechnical fields) has indirectly contributed to the phenomenon of increasing antibiotic resistance.

Thus, the correct and judicious administration of antibiotics is extremely important, because microorganisms have a phenotypic and genotypic adaptability and can easily become resistant to the action of the chemotherapeutic. Excessive or misuse of antibiotics has led to the selection of microorganisms that have developed mutations that give them resistance to antibiotics. Bacterial resistance to antibiotics has been and remains an important therapeutic issue.

An antibiotic does not have an universal effect against all types of bacteria, just as an antiviral is not universal against all viruses (Pitout J.D.D, et al. 1998, Martinez J.L., 2012 Romeo Teodor C., Moruzi R.F., 2020).

Statistical data show that annually, worldwide, more than 20 million people lose their lives through infectious diseases, as a result of the fact that antibiotics have gradually lost some of their initial effectiveness.

This resistance to antibiotics can be natural or acquired, the latter being generalized in several planes such as genetic resistance or a biochemical resistance (Burduniuc O. 2012,Muntean D., Licker M., 2019).

The aim of this paper is to analyse the resistance of some Enterobacteriaceae to antibiotics, their identification by specific cultural methods, the evaluation of antibiotic resistance and the detection of resistance mechanisms.

MATERIALS AND METHODS

The data of this study were collected from a private clinical laboratory for medical tests. Biological samples were taken from patients hospitalized to a hospital for chronic and palliative diseases, aged between 55 and 98 years. The period in which biological samples were collected, identified and analyzed, from a microbiological point of view have been for 3 months, respectively January, February and March 2020.

The working methods used are in accordance with the CLSI-Clinical and Laboratory Standards Institute and the EUCAST norms - European Committee on Antimicrobial Susceptibility Testing. Isolation, enumeration and presumptive identification of Enterobacteria from urine, was performed on selective culture medium differential CLED-agar (cystine-lactose-electrolyte deficient), followed by biochemical identification using MIU culture media (mobility indole urea), TSI (Triple Sugar Iron Agar), Simmons and at the same time with the help of indole and hydrogen sulfide strips (Table 1).

In order to evaluate the antibiotic sensitivity of the identified bacteria in the biological samples, antibiograms were made with discs impregnated with a known amount of antibiotic (Bio-Rad, France).

The principle of the antibiogram involves the cultivation of bacteria in vitro, under standard conditions, in the presence of known amounts of antibiotic (CLSI 2020, EUCAST 2013)

A number of factors can influence the results of an antibiogram, the most important being: the culture medium, the studied microorganism, the used antibiotics, the temperature and duration of incubation, the used technique and the interpretation criteria (Doma A.O., 2015).

Thus, two phenomena occur at the same time: the growth of pathogenic bacteria and the spread of the antibiotic. After 16-18 hours at 37 °C, the antibiogram is read and interpreted, the diameter of the complete inhibition zones being measured with the subler. Then, the report to the interpretive table is made (CLSI 2020), the antibiotics being grouped in sensitivity categories for each antimicrobial active substance (sensitive, intermediate or antibiotic

resistance). The results of the test allowed the selection of the antibiotic that shows the most intense bactericidal activity. This antibiotic will be administered to the patient to obtain a maximum therapeutic effect, identifying some resistance behaviors of bacteria.

The circumference of the inhibition zone is established from the first hours of incubation as the geometric place of the points where the antibiotic reached the minimum inhibitory concentration (MIC) at the critical moment of the culture (lag phase and first 2-4 generations). Thus, the diameter of the inhibition zone varies inversely with MIC (Buiuc D., et al. 2017, Muntean et al., 2019).

The classification of the tested bacterium into categories: sensitive, intermediate or resistant to antibiotics is done by one of the following techniques: a- comparison of the diameters of the inhibition zones obtained with a certain amount of antimicrobial substance deposited on the line that separates the tested strain from a sensitive reference strain, seeded on the same plate and b-reporting to interpretive tables. Such tables are compiled from the MIC spread point graph versus the diameter of the inhibition zones versus 100-150 selected strains to comprise the range of clinically significant MICs. The regression line is calculated and plotted for each antibiotic and rupture points are designed of MICs on the diameter of the inhibition zones.Table 2 also shows the breaking points of the MIC (CLSI, 30th edition, 2020).

The choice of antibiotics tested in antibiograms takes into account: the natural resistance of bacteria, the location of the infection and is done so as to reduce the risk of multidrug resistance in isolated strains from hospitalized patients.To test the beta-lactamine sensitivity of Enterobacteriaceae, the following variants are practiced (Buiuc D., et al. 2017, Muntean D., 2019, CLSI 2020):

Aminopenicillins: Ampicillin (10 µg)

Ureidopenicillin: Piperacillin (100 µg)

Cephalosporins 1st Generation: Cefazolin (30 µg)

Cephalosporins 2nd Generation: Cefuroxime (30 µg)

Cephalosporins 3rd Generation:Ceftazidime (30 µg), Cefotaxime (30 µg) or Ceftriaxone (30 µg)

Cephalosporins 4th Generation: Cefepim (30 µg)

Carbapenems: Imipenem (10 µg), Meropenem (10 µg)

Beta-lactamine + IBL: Amoxicillin + clavulanic acid (20/10 µg) or Ampicillin + sulbactam (10/10 µg); Piperacillin + tazobactam (100/10 µg); Ticarcillin + clavulanic acid (75/10 µg).

Table 1. Reaction of bacteria to TSI, MIU andSimons culture media

Sample	TSI medium				MIU medium			Simmons
	Glucose	Lactose	Sucrose	H_2S	Indol	Mobility	Urease	Citrate
E. coli	+	+	+	-	+	+	-	-
Klebsiella spp.	+	+	+	-	+/-	-	+	+
Proteus spp.	+	-	-	+	+(*P.vulgaris*) -	+	+	+

Table 2. Interpretative categories of inhibition diameter and of MIC (CLSI, 30th edition, 2020)

Antimicrobial agent	The contents of the disc (µg)	Interpretive categories and breaking points with the diameter of the area (mm)			Interpretive categories and breaking points with the diameter of CMI		
		S	I	R	S	I	R
Ampicillin	10	17	14-16	13	8	16	32
Amoxicillin	-	-	-	-	2	4	8
Piperacillin / tazobactam	100/10	21	18-20	17	16/4	32/4-64/4	128/4
Amoxicillin/ Clavulanic acid	20/10	18	14-17	13	8/4	16/8	32/16
Cefazolin A	30	23	20-22	19	2	4	8
Cefazolin U	30	15	-	14	16	-	32
Ceftriaxone	30	23	20-22	19	1	2	4
Ceftazidime	30	21	18-20	17	4	8	16
Cefuroxime	30	23	15-22	14	4	8-16	32
Imipenem	10	23	20-22	19	1	2	4
Meropenem	10	23	20-22	19	1	2	4
Gentamicine	10	15	13-14	12	4	8	16
Amikacin	30	17	15-16	14	16	32	64
Ciprofloxacin	5	26	22-25	21	0.25	0.5	1
Levofloxacin	5	21	17-20	16	0.5	1	2
itrofurantoin	300	17	15-16	14	32	64	128

RESULTS AND DISCUSSIONS

From the total samples of patients from the investigated group, only 83 patients were reported with the presence of some species of *Enterobacteria*. From the total antibiograms evaluated, in the medical analysis laboratory, in January, February and March 2020, the number of female patients was higher (67.34%) than that of men (32.65%) (Fig. 1).

Therefore, among the representatives of the *Enterobacteriaceae* family, the presence in the samples analyzed according to their frequency of *Escherichia coli* in 51%, *Proteus mirabilis* 30% and *Klebsiella spp*. 19% was reported (Fig. 2).

Analysis of *Escherichia coli* strains identified in the analyzed biological samples

*Escherichia coli*is the etiological agent of urinary tract infections, in a percentage of 75% and even up to 95%, and some strains of *E. coli* can cause kidney failure. Laboratory results have shown in the case of these infections the definite sensitivity of *E. coli* at: amikacin, imipenem, piperacillin / tazobactam and meropenem

From the total of the identified samples (42), it was found the resistance of: 32 strains in the presence of amoxicillin and ampicillin, 20strains to biseptol, 19strains for cefazolin and cefuroxime, 17strains with resistance to amoxicillin / clavulanic acid, gentamicin, ciprofloxacin and ceftriaxone, 15strains resistant to levofloxacin, 13strains resistant to ceftazidime and 12 strains with resistance to nitrofurantoin (Fig. 3).

Analysis of *Proteus mirabilis* strains

The bacteria of *Proteus mirabilis* have a special medical importance, occupying the second place in the etiology of urinary tract infections, after *Escherichia coli*. *Proteus mirabilis* can cause urinary tract infections, gastroenteritis, but can also cause localized infections, usually in surgical wounds in the abdomen or even sepsis, especially in elderly patients. However, urinary tract infections represented by cystitis remain in the first place.To patients with urinary stones, *Proteus mirabilis* is often isolated in the urine thanks to specific recurrent bacteriuria (Schaffer JN et al., 2015, Buiuc D. and Negrut M., 2017).From the analysis of antibiograms, the sensitivity of *Proteus mirabilis*was found to the following antibiotics schows in the Fig. 4.

Analysis of *Klebsiella spp.* strains

From the analysis of the graph regarding the monitoring of antibiotic resistance of *Klebsiellapneumoniae* strains, it is obvious that these bacteria have a resistance to penicillins, fluoroquinolones and cephalosporins.

The sensitivity to amikacin, imipenem, meropenem and piperacillin / tazobactam, of *Klebsiella spp*. strains is varied, being presented in the Fig. 5.

Analyzing the antibiotic resistance of the Enterobacteria identified in the analyzed biological samples, we found that it varies to the groups of antibiotics tested, depending on the identified strain, but according to the favoring factors. ESBL production results in resistance to ampicillin, amoxicillin and cephalosporins. For this reason, these antibiotics have been associated with beta-lactamase inhibitors.

The presence of 38 beta-lactamase-producing strains with broad spectrum was observed, namely 45.78% of the total strains of isolated Enterobacteriaceae(83) (Fig. 6). The samples analyzed in this study showed in the order of the frequency of BLSEproducing strains, a percentage of 62.5% in *Klebsiellaspp*, 60% in *Proteus mirabilis* and 30.95% in *E. coli*.

The ESBLproduction results in resistance to ampicillin, amoxicillin, and cephalosporins. For this reason, these antibiotics have been associated with beta-lactamase inhibitors, e.g.amoxicillin and clavulanic acid from Augmentin or piperacillin + tazobactam. Most Enterobacteria, including those of BLSE producing, are sensitive to carbapenems (imipenem, meropenem). These antibiotics are preferred in medical practice, considered as a reserve. The emergence of new β-lactamases with direct hydrolysis activity of carbapenem, has contributed to an increased prevalence of Enterobacteriaceae, resistant to carbapenem (Paterson D.L., Bonomo R.A., 2005, Doma A. O., et al. 2015).

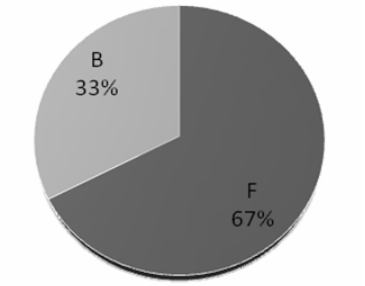

Fig. 1. Distribution by sex (%) of the analyzed samples

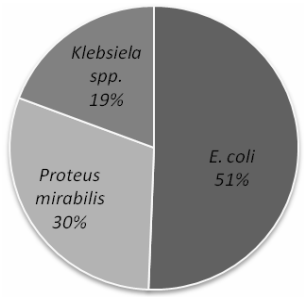

Fig. 2. The main bacterial strains (%) identified in biological samples

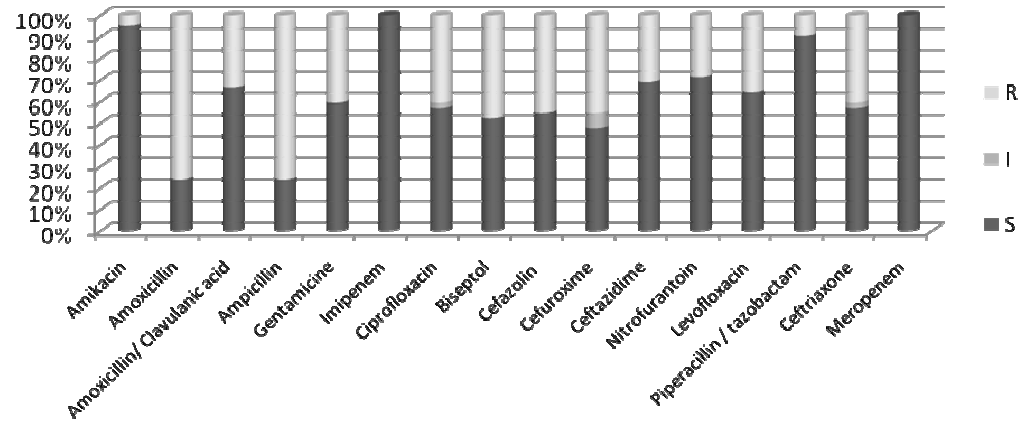

Fig .3. Antibiogram chart with *Escherichia coli*

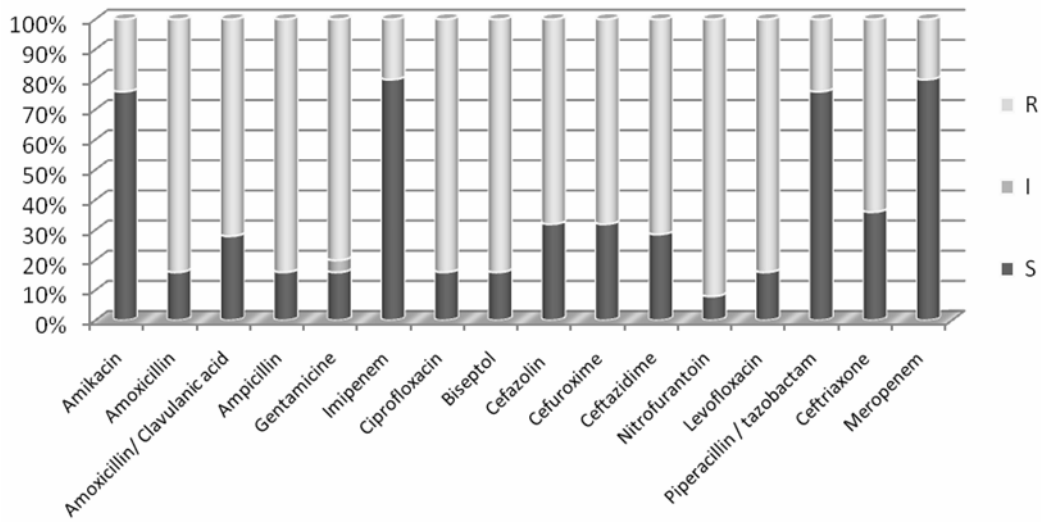

Fig. 4. Antibiogram chart with *Proteus mirabilis*

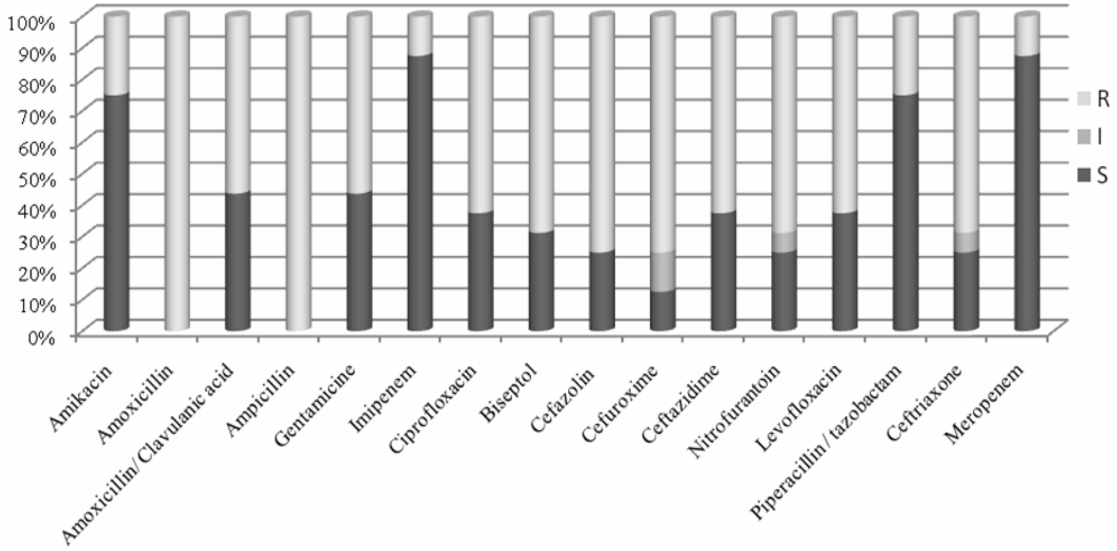

Fig. 5. Antibiogram chart with *Klebsiella spp.*

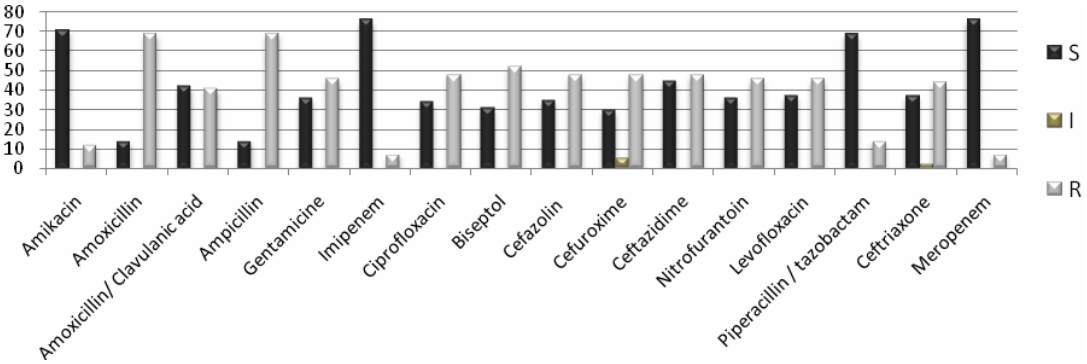

Fig. 6. Graph of Enterobacterial resistance to antibiotics

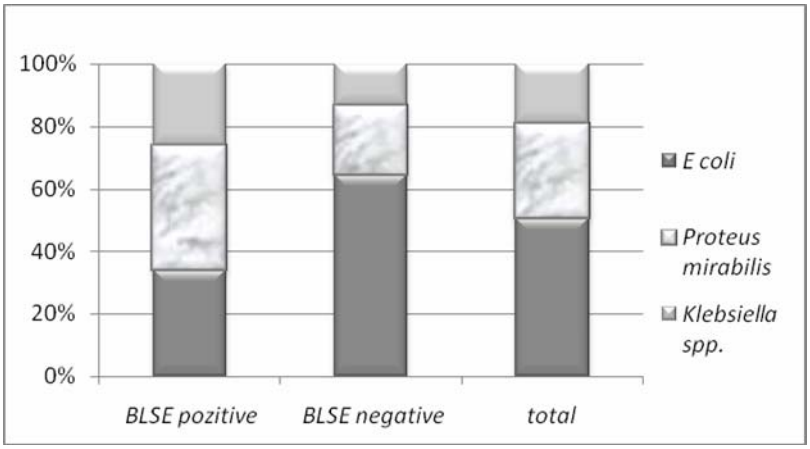

Fig. 7. Evaluation of BLSE-producing Enterobacteriaceae

CONCLUSIONS

The results of the antibiograms showed a definite sensitivity of *E. coli* bacteria to: Amikacin, imipenem, piperacillin / tazobactam and meropenem.
Regarding to antibiograms on *Proteus mirabilis* cultures, bacterial resistance to most antibiotics tested was observed. The only antibiotics that showed sensitivity to the bacteria tested were: amikacin, piperacillin / tazobactam, imipenem and meropenem. *Proteus mirabilis* strains have been shown to be largely resistant to amoxicillin, amoxicillin / clavulanic acid, ampicillin, gentamicin, ciprofloxacin, biseptol, cefazolin, cefuroxime, ceftazidime, nitrofurantoin, levofloxacin and ceftriaxone.
Klebsiella species are resistant to penicillins, fluoroquinolones and cephalosporins. On the other side, the sensitivity of the tested *Klebsiella* strains to amikacin, imipenem, meropenem and piperacillin / tazobactam was observed.
In medical practice, antibiotics have been and stillare among the most prescribed pharmaceutical preparations. In recent years, a progressive increase in multidrug-resistant microorganisms has been remarked, on the one hand by β-lactamases acquiring or by the simultaneous presence of other resistance mechanisms(Pitout J.D.D., 1998, Drieux L., et al. 2008, Doma A. O., et al. 2015).
Researches and data on bacterial resistance to antibiotics are important, primarily for choosing the right practical therapy. It is important in making final decisions, to corroborate laboratory tests with biochemical, immunological etc. The clinical use of antibiotics must coincide and has to be based on principles that must ensure the efficiency and safety of administration (Canton R., et al. 2008, Romeo T.C., et al 2020).

ABSTRACT

This paper, which proposed a study of antibiotic resistance of Enterobacteriaceae is based on analyzes and statistical processing that were performed from January 2020 to March 2020.
It is worrying that 45.78% of all isolated strains are ESBLproducing, which means that bacterial resistance is increasing and their sensitivity is getting lower and lower.
Most Enterobacteria, including those of ESBLproducing, are sensitive to carbapenems (imipenem, meropenem). These antibiotics are preferred in medical practice, considered as a reserve.

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