"Batum Shota Rustaveli State University"

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Identification of E.coli, which causes nosocomial infections in Adjara, and study of the antibiotic resistance profile

Academic degree of the Ph.D

To obtain a dissertation annotation

Specialty: Microbiology

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Introduction

Relevance of the research topic:

Antibiotic resistance is one of the most important and global health problems, as it quickly spreads and increases the types of microorganisms, which are resistant to several antibiotics (Jacopin .... 2020; Khan 2020). Formation and distribution of sustainable strains, as well as incorrect use of antibiotics among people, unsatisfactory hygienic and sanitary conditions complicate control over health care infection, as well as in the field of animal husbandry and poultry farming (Yang 2009, Boonyasiri …2014).

In the fight against antibiotic-resistant, the main directions are: the organization of monitoring of the system, which determines the circulation of sustainable microorganisms and their gene resistance, development and implementation of a single standardized methodology to determine the sensitivity of microorganisms to antibiotics. Criteria for knowledge of resistance mechanisms and interaction based on pharmacodynamics-pharmacokinetics make it possible to improve, make effective monitoring not only at the level of individual hospitals, but also in the region and in the country. On the other hand, the problem of antibiotic resistance is poly-resistance to the global spread of microorganisms, this contributes to globalization, which is the result of mass migration of the population in different countries.

In the modern world, a patient who is located in the hospital in another country of the world may apply to the doctor. Patient "International" This may be a person who is forced to live in another country due to natural disasters; as well as a tourist who is hospitalized while traveling due to accidents; The patient who receives medical care medical tourism is cheaper than in his country (dentistry, ophthalmology, etc.).

Hospitalization in the hospital in another country is accompanied by a risk of infection with poly resistant microorganisms that move from the patient to the patient from one

country to another, where they did not apply earlier. It is clear that monitoring of such microorganisms should be conducted at the international level(Antimicrobial Resistance… 2014; Singh… 2012).

Along with other microbes, antibiotic-resistant strains were observed in enterobacteria, especially in relation to beta-lactam. One of the most common and clinically important mechanisms (MOGLAD ..... 2020) in the formation of the resistance of enterobacteria against beta lactam antibiotics is the beta lactamase ESBL

ESBL is an enzyme that causes resistance to β-lactam antibiotics. Among them: Penicillin, cephalosporins, monobactams. Infections caused by bacteria are characterized by frequent mortal cases (Hashemi B .... 2018). In different countries of the world, antimicrobial resistance to the third generation cephalosporins was recorded.

One of the best examples of antibiotic resistance is poly resistant to drugs, and ESBL producing *Escherichia coli*, which can cause life-threatening infection (Pormhammad ... 2019). It is true that *E. coli* is part of a human intestinal flora, but it can cause diseases such as urinary tract diseases and diseases of the central nervous system.

Animals are an important *E. coli* tank, and the use of antibiotics in these animals is an *E. coli* antibiotic resistance source for people, as it is transmitted to man through direct and indirect contact. To begin effective treatment of infection, it is necessary to have information not only about the distribution of this infection, but also the sensitivity of infection to antibiotics.

Based on the above, to identify the formation mechanisms of resistant bacteria are of great importance to develop methods to combat antibiotic resistant strains.

Structure and dissertation of thesis:

The dissertation contains 3 chapters: literary reviews, research facilities and methods, experimental part, conclusions and used literature. (List of tables -12, charts -8, images -21, antibiotics used in the study; primers used in the study; literature 104).

Objectives and purpose

Based on the foregoing, the purpose of our research was the detection of nosocomial infections caused by *E.coli*, their identification, detection, molecular genetic substantiation of resistance

The following objectives were pushed in dissertation:

- Collecting materials from nosocomial infections from suspicious patients;
- Phenotypic study of antibiotic resistant strains and determining the reasons for resistance;
- Bacteriological examination of the sample to determine the possible bonds of the disease in the material;
- Creating an Isolate Bank for further molecular genetic studies;
- Antibiotic gram, microbe sensitivity determination;
- Identification of the production of β-lactamase (ESBL) of a wide range of action;
- DNA release from culture and study of the genetic profile in order to detect resistance.

The object of research and methodology

As an object for the study was taken the causativeagent of nosocomial infections -*E. coli*

Material for research was examination of immunocompromising patients in the intensive care unit for infections of the respiratory tract, intra-abdominal organs, skin and soft tissues, urinary tract and blood after 48 hours or more (total 540): sputum, urine, wound swabs, blood, venous material and material from bladder, from the surface of the catheter tip.

A retrospective study of various biological samples was carried out. The bacteria were isolated and identified by standard bacteriological methods, namely by sampling from the appropriate nutrient zones and then by separating the pure culture. Finally, cultures were identified using the API test, and antibiotic susceptibility was determined by Kirby-Bauer diffusion and E-test. The double disc method was used to determine the producers of *E. coli* broad spectrum beta-lactamases (ESBL).

A total of 540 samples were examined, of these, 26 were resistant, and 22 were susceptible to antibiotics, of the latter found the ISBL produce.

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Sustainable strain genotypes were studied using polymerase chain reaction methods (RAPID-PCR, Multilex PCR) and gel electrophoresis methods.

Genes of the class ESBL TEM, ESBL SHV and CTX-M were found in these samples as a result of molecular studies of genes causing phenotypic genotypic correlation and resistance.

The multiresistance genes detection have been carried out by the reversehybridization method.

Based on the results of the study, pathogenic strains of E.coli showed 100% resistance to the following antibiotics: FEP-cefepim, CXM-cephaloxime, CRO-cephalaxone, CAZ-Ceftazidim, CTX-Cefotaxime, ATM-Astreonam and AMP-Ampicillin. It should also be noted that the vast majority of the above antibiotics are beta-lactam antibiotics in the broad spectrum and the reason for their resistance is the ability of E.coli to produce beta-lactamase in the extended spectrum of ESBL-extended spectrum. It is known that the mastic gene for beta lactamase is located in the plasmid and is easily spread. As for carbapenems, as mentioned above, all isolates were resistant to ATM-aztreonam, while about 40% of isolates against IPM-imipenem and about 77% with respect to MEM-meropenem maintained efficacy.

As a result of our study, CIP-ciprofloxacin had a resistance of up to 70% in *E.coli* isolates, with the same rate of LVX-levofloxacin, AMC-amoxacillin, DOX-doxycycline, and SXT-trimethoprim/sulfamoxazole.

From aminoglycosides - AMK-amikacin was relatively effective and 73% of isolants were sensitive to it, and in the case of gentamicin only 42%.

Based on our research, it can be said that only four antibiotics can be used as effectively as possible against *E.coli*, such as CST-colistin (almost 100% susceptibility), PIP/TZP-piperacillin/tazobactam (88%) , IPM and MEM meropenem 85% sensitivity.

Molecular-genetic research has revealed CTX and KPC genes in strains of *E.coli* that cause reproductive nosocomial infection in ESBL. In phenotypic-resistant strains with penicillins, 3rd and 4th generation cephalosporins, and inhibitors, CTX, TEM, and SHV genes were detected in phenotypic-resistant strains, and in two samples with wild genes, ESBL class TEM type mutant genes were observed: TEM AS 104 E, TEM AS 238 .

Scientific news:

- For the first time in Adjara, a study was conducted in the dissemination of nosocomial infections caused by *E. coli;*
- It was determined by the role of *E. coli* in nosocomial infection among the grams of negative bacteria.
- The data were obtained from nosocomial strains registered in the intensive care and therapeutic departments sensitive to antibiotics.
- For the first time in the Adjara region, the circulation of *E.coli* resistance genes was determined.

Scientific and practical value

Considering the data obtained of ESBL-reproductive *E. coli*, will be given a recommendation for the National Center for Disease Control detect all diagnostic strains for the production of β-lactamase;

The most effective antibiotics for *E. coli*-resistant strains have been identified, observed in resuscitation and intensive-therapeutic departments of Adjara hospitals;

Antibiotics have been identified ,but have lost their effectiveness at this stage in relation to E. coli, which cause of nosocomial infection; This information will be provided to both the National Center for Disease Control and doctors in hospitals in the Adjara region;

. Also, the data will be transferred to the relevant health services for the development of etiotropic and empirical therapeutic measures.

Microbiological research methods:

- ESBL confirmation method;
- Dual disk synergy test (DDST) ;
- KarbaBauer's disk diffusion method ;
- E-TEST system.

Research on biochemical methods:

- Primary biochemical tests;
- Biochemical test for API20E microbial identification.

Molecular methods of research:

- DNA-extraction;
- Polymerase Chain Reaction Method (PCR);
- Electrophoresis in Agarose gel;
- Revers hybridization method.

Literary Review

Nosocomial infection (Latin: nosocomium - hospital, Greek word: nosokomeo - care for the sick). According to the European Regional Bureau of the World Health Organization, nosocomial infection is any clinical infectious disease that develops in the patient during treatment or care. Also, any infectious disease of the hospital staff, developed as a result of its work in the same building, which has nothing to do with the detection of symptoms (being

in the hospital / or not). Infections are considered to be hospitalized if they develop after at least 48 hours of hospital admission (excluding cases where the patient is admitted to a medical facility during the incubation period of infectious disease, the duration of which is not more than 48 hours).Nosocomial infections are a serious medical-social, economic, and legal problem in individual intensive care.

Microorganisms are considered to be the source of nosocomial infections that have adapted to existing conditions during long-term stay in the hospital. It can be fungi, bacteria, and viruses. In most of Georgia's multifunctional inpatients, bacteria are found to cause nosocomial infections, although their percentage is within the area of the hospital. It depends on the population of patients, the localization of infection, the routine practice of using antibiotics, the infectious control of the methods used, etc. (Vincent..2003; T. Koiava... 2017). Gram-negative bacteria have been among the leading causes of nosocomial infection in hospitals in different countries in recent years. Patients who undergo a course of treatment in the urological department, as well as in patients with resuscitation and intensive care, are particularly often infected with *E.coli* infection (Stephen..2001). Frequent causes of infection in patients are the use of diagnostic and medical devices, as well as low hygiene of medpersonal hands. Cases of ventilator-associated pneumonia have been investigated, as well as blood infections associated with central intravenous catheters in urinary tract infections. The main cause of urinary tract infections is *E. coli* . In a number of other studies, *E. coli* was not found to be the predominant causative bacterium, although its share was quite significant. Noteworthy is the localization of caused by *E. coli* in different places: lower respiratory and infections of urinary tract, skin and soft tissues, intraabdominal, ect. (Arndt ...2011).

Resuscitationand Intensive Care Departments differ from other multifunctional inpatient subjects in that it is here that there is constant contact between the patient and the medicalpersons. In addition, patients are subjected to numerous invasive procedures. Prolonged use of antimicrobial drugs in these departments creates conditions for the selection of strains that have developed resistance mechanisms to antibiotics. This increases the resistance of previously sensitive gram-negative bacteria. For example, such as E.*coli,*

Klebsiella spp, Acinetobacter Bauman, Pseudomonas aeruginosa, Citrobacter spp. Most of all, these microbes are the cause of infections in the hospital.

The problem of combating the causes of nosocomial infections has long been missed within a particular country and has been acquired worldwide. This is evidenced by the 2001 Janmo-Smier designed global trend, which emphasizes the need to decipher molecular mechanisms of resistance in order to create new means of diagnosing resistance. Studies in this area are intensive. Therefore, the study of the prevalence of these infections is a study of susceptibility to antibiotics, and the subsequent determination of the causes of antibiotic resistance is important and does not lose relevance. Like other countries, Georgia has faced rapid spread of nosocomial infection. The Georgian government has issued an ordinance on №29 on January 11, 2017, based on the issue of the alarming rise of antibiotic-resistant strains, on the basis of which the 2017-2020 National Strategy for anti-microbial resistance was published.

Results and their judgment

In the clinics of the Adjara region, in order to determine the etiology of nosocomial infections caused by *E.coli* and to study the profile of antibiotic resistance, the study was conducted in two stages:

- Separation of *E.coli* from biological materials and phenotypic detection of antibiotic resistance ;
- Detection of resistance-encoding genes.

The experiment was conducted mainly in samples taken from suspected patients on nosocomial infection of three multipurpose clinics in the city of Batumi. Patients in the clinic are the highest risk groups for infection with nosocomial pathogens due to constant contact with medical staff and a large number of invasive procedures. An additional factor may be antibiotic therapy, which is used almost regularly against these patients, which contributes to the selection of antibiotic-resistant strains. Since *E.coli* is associated with many different human infections, pathogen detection is possible in different biological materials. We isolated the strains of *E.coli* from the biological material: urine, blood, liqueur,

sputum, and smear from the wound. Washes from central vein and urine catheters were also used.

Results of E.coli antibiotic resistance study

In the first stage of the study, we performed phenotypic detection of antibiotic sensitivity. The experiment included isolating and identifying bacteria using standard bacteriological methods: sowing samples in food areas, then isolating and identifying pure culture. Sensitivity to *E.coli* antibiotics and screening for a wide range of β-lactamase derivatives (ESBL) have been studied.

The study, conducted by us, examined 540 samples. Of these samples, 236 were weighted, 82 were identified as gram-positive bacteria, and the remaining 222 samples were detected by gram-negative bacilli. From these specimens came sputum - 89, urine - 47, biological fluid-exudates, the tip of the vein and bladder catheters - 22, smear from wound - 12, blood - 52. Of the 222 samples studied*, E.coli* was identified and identified from 47, of which 26 were found to be multiresistant, accounting for 55% of the allocated *E.coli*. Studies have shown that *E.coli* accounts for 21% of the population of gram-negative bacteria and 12% of the resistant *E.coli* population. *E. coli* was sampled as follows: urine - 6 strains (12.8%), sputum - 12 (13.5%), blood -2 (3.9%), smear from wound -1 (4.5%), exudates -5 (22.7%) (Table 8).

Table 8. E.coli is extracted from samples

Name of biological material	**Total number of samples examined**	**Results of *E.coli* seeding**	**Number % E.*Coli* seeding**
Urine	47	6	12,8
Sputum	89	12	13,5
Blood	52	2	3,9
Smear from wound	12	1	4,5
Biological fluids taken from the	22	5	22,7

catheter			

Quantitatively, the identification of *E.coli* predominates over sputum samples, but at the percentage ratio *E.coli* predominates in the prevalence of biological fluids (Figure 1). These samples are taken from catheters, which may be related to *E.coli's* ability to form biogas.

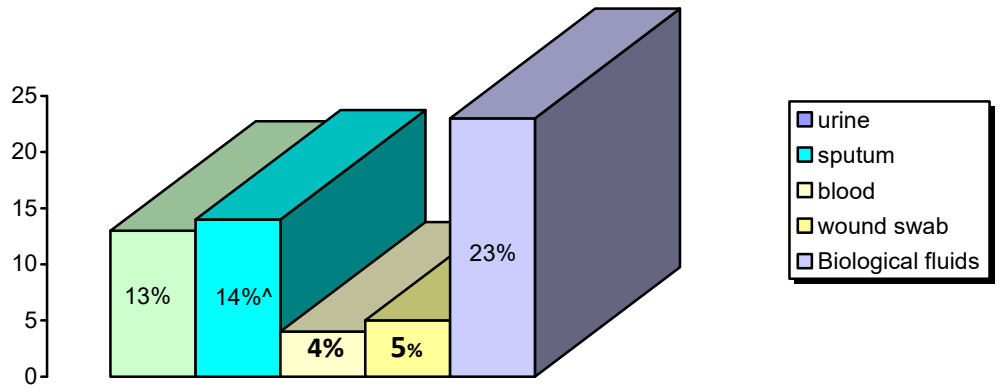


Diagram 1. % Ratio to various biological materials of seeding cultures.

Microbiological analysis of antibiotic resistance of samples

As a result of phenotypic studies of antibiotic resistance, the use of the disc-diffusion method and ESBL-proof tests has been shown to be quite severe in Adjara, in terms of *E.coli*-resistant strains in terms of detection. Antibiotic sensitivity has been studied using a disc-diffuse method where different antibiotic groups and generations have been used. The susceptibility of the microorganism was calculated in accordance with the European-EUCAST (European Commission on Antimicrobial Susceptibility Testing) and the Statute of the American Committee -CLSI. ESBL production is known to be directly related to the multifunctionality of the micro-organism. Phenotypic scrin was therefore performed to produce beta-lactamase. Table №9 lists the antibiotics used to determine the antibiotic sensitivity of *E.coli.*

Table 9. List of antibiotics and abbreviations

N	Abbreviation	Antibiotics

1	CST	Colistin
2	FEP	Cefepime
3	CXM	Cefuroxime
4	CRO	Ceftriaxone
5	CAZ	Ceftazidime
6	CTX	Cefotaxime
7	PIP/TAZ	Piperacillin/tazobactam,
8	CIP	Ciprofloxacin
9	LVX	Levofloxacin
10	IPM	Imipenem
11	ATM	Aztreonam
12	AMK	Amikacin
13	AMP	Ampicillin
14	AMC	Amoxicillin/clavulanic acid
15	SXT	Trimethoprim/sulfamethoxazole
16	DOX	Doxycycline
17	MEM	Meropenem
18	GEN	Gentamicin

The antibiotic sensitivity test clearly showed a high resistance of *E.coli* to the antibiotics used (pic. 1). 16).

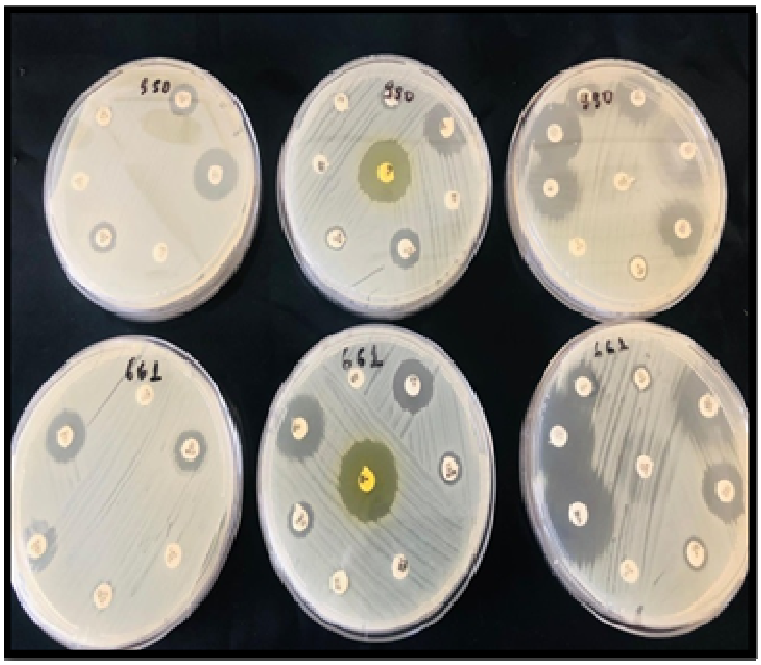


Figure 16. E. Coli's antibiotic sensitivity

As part of our study, all strains that showed resistance to β-lactam antibiotics were tested for β-lactamase production (ESBL) with a double disc synergy test. As can be seen from the picture (pic. 17), the inhibition zone between third-generation cephalosporin and clavulanic acid discs indicates the presence of ESBL in the study strains. As a result of our screening of 26 resistant strains that showed resistance to cephalosporins, 23 strains were found to reproduce ESBL, accounting for 88% of the total number (pic1). Because most of the isolators were ESBL reproducers and only 3 isolates were the exception, and this was such a small amount that comparing the antibiotic-sensitive profile between the ESBL reproducer and the ESBL non-producer would not be valid and unfulfilled. It should be noted that the antibiotic resistance of ESBL non-productive isolates was also quite high.

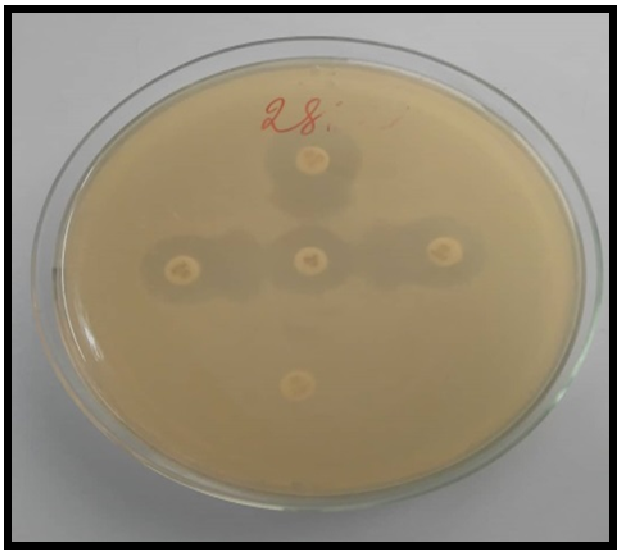


Figure 17. Double disc synergy test

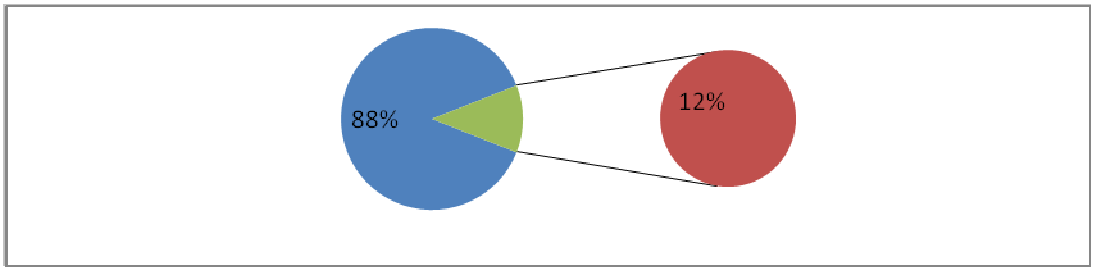


Diagram 1. Positive and negative profile of ESBL reproducing E.coli

We also used a modified version of the disc-diffuse method for determining antibiotic susceptibility in the form of E-test. An E-test strip with a gradient of different concentrations of antibiotics was placed on a dense food is on the surface, with an inoculated research strain. After 18-24 hours of incubation, an ovalzone of growth suppression was created. Depending on the location of the intersection with this zone, the result was determined by the minimum inhibition zone. E-test was used to determine Colistin susceptibility. All test strains showed sensitivity to this backup antibiotic.

In a detailed review of each isolate, it was found that 83% of urinary isolates were resistant to CIP-ciprofloxacin, LVX-levofloxacin, and DOX-doxycycline, 67% resistant to AMC-amoxicillin/clavulanic acid and SXT-trimethoprim. The highest susceptibility of *E.coli* isolates (100%) was observed to CST-colistin, IPM-imipenem, and MEM-meropenem, while

PIP/TZP-piperacillin/tazobactam (83%) and 67% compared to AMK-amikacin relatively less (diagram 2).

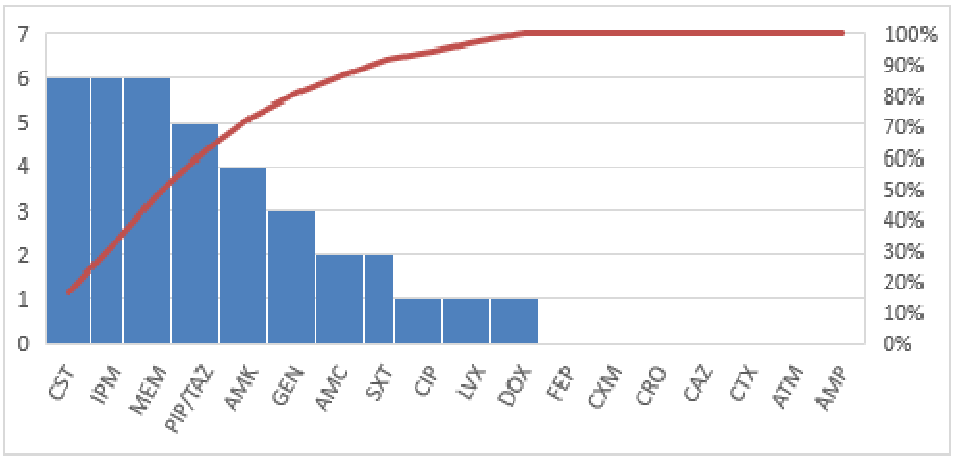


Diagram 2. Antibiotic sensitivity of E.coli separated from urine samples

Isolates of intestinal sticks separated from sputum have been found to be highly resistant to 7 antibiotics. In particular, 100% resistance to the following antibiotics was detected: FEP, CXM, CRO, CAZ, CTX, ATM, AMP. Less than 30% sensitivity was observed to CIP,SXT, and GEN,less then 50% sensitivity DOX,LVX,AMC, sensitivity of AMK is 50%, as to CST,PIP/TZP and carbopenems IPM ,MEM the opposite was observed 100% and 90% sensitivity (diagram 3.)

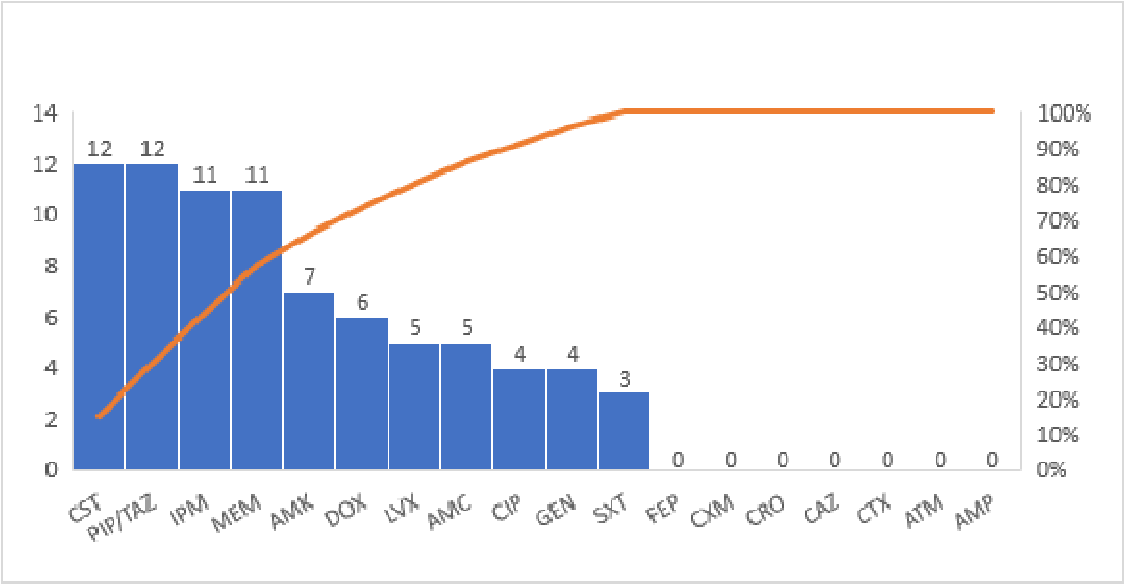


Diagram 3. E.coli separated from sputum specimens

A study of isolates obtained from the discharge of vein and bladder catheters found that *E.coli* is resistant to 7 antibiotics. In particular, 100% resistance to the following antibiotics was detected: FEP, CXM, CRO, CAZ, CTX and AMP. 80% resistance was observed to CIP, LVX, AMC, SXT and DOX. 40% sensitivity to IPM ,MEM 100% sensitivity to E.coli was maintained in only two antibiotics - CST, and AMK.80% sensitivity just to PIP/TZP antibiotic(diagram 4).

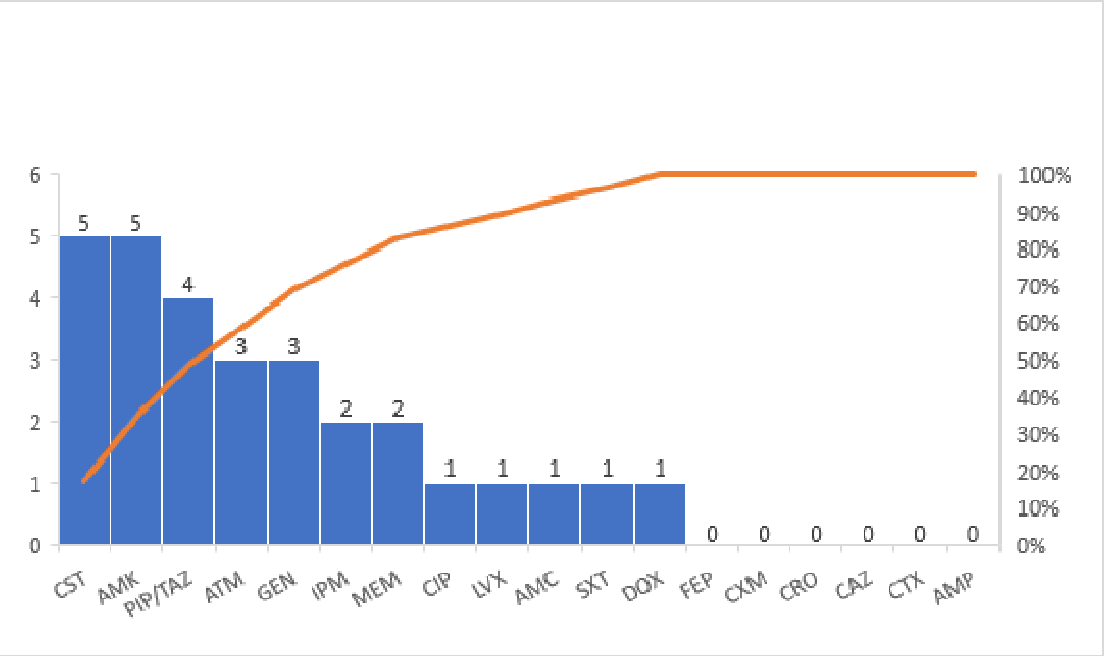


Diagram 4. Antibiotic sensitivity of E.coli separated from catheter runoff samples

As for blood-identified isolates, only two showed resistance to all representatives of cephalosporins FEP, CXM, CRO, CAZ, CTX, CIP, LVX, ATM, AMP, SXT, DOX and GEN One blood isolat showed 100% sensitivity to colistin and carbopenem(IMP ,MEM) and a 40% susceptibility to piperacillin/tazobactam, amikacin and amoxicillin (diagram5).

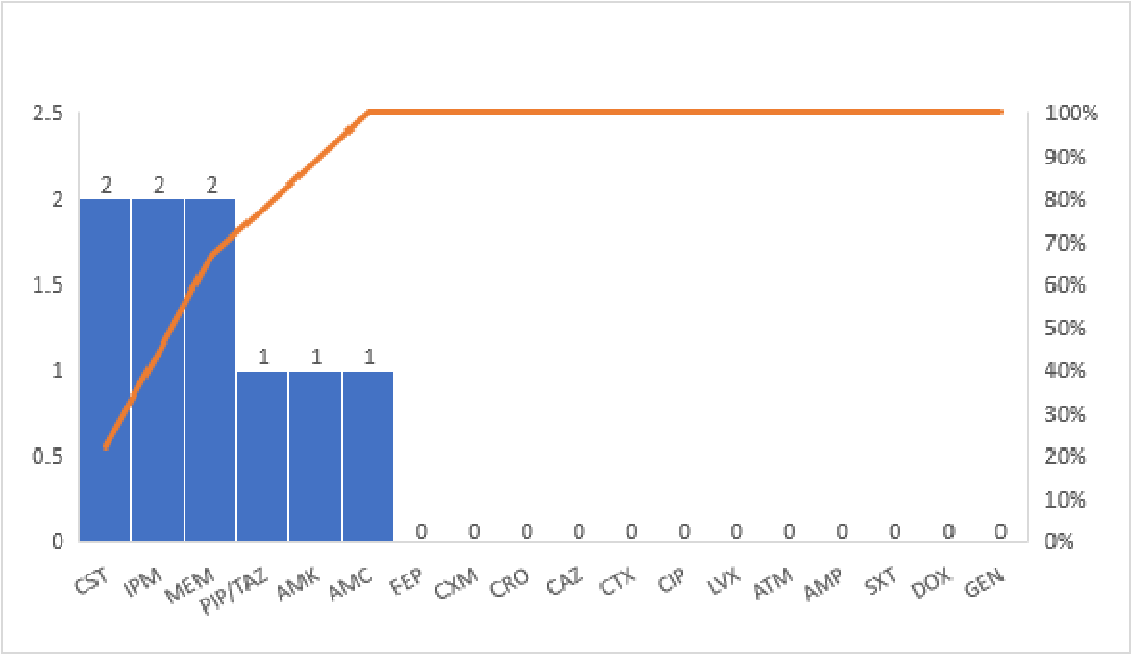


Diagram 5. Antibiotic sensitivity of E.coli separated from blood samples

One isolator identified from the wound smear revealed multirection. In particular, it was found to be resistant to the following antibiotics: FEP, CXM, CRO, CAZ, CTX and also ATM, AMP, SXT, DOX. And the susceptibility to the following antibiotics - CST, PEP/TAZ, CIP, LVX, IPM, AMK, AMC, MEM and GEN was maintained at 90% (diagram 6).

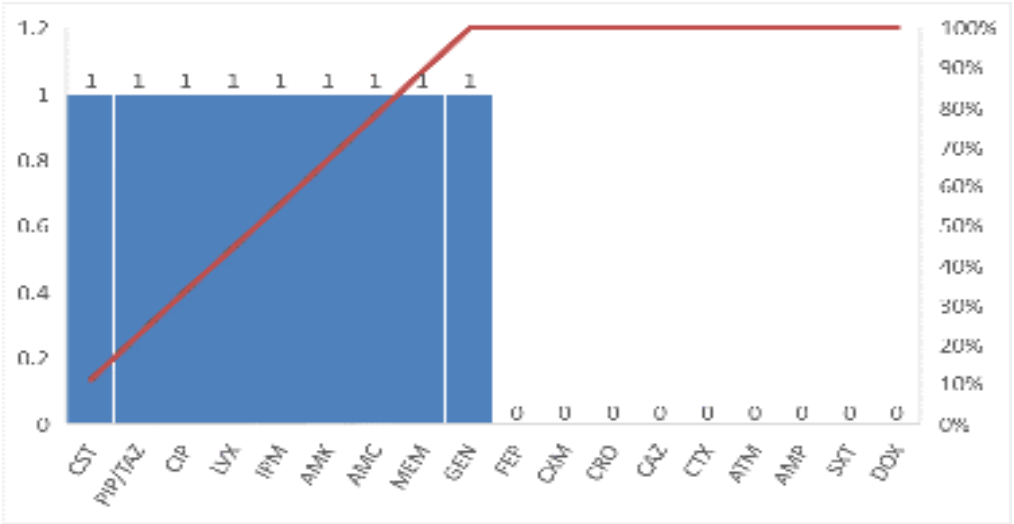


Diagram 6. Antibiotic sensitivity of E.coli separated from smear wound samples

Thus, the antibiotic resistance of *E.coli* isolated in the resuscitation departments of Adjara medical institutions was quite high. If we consider all kinds of samples together, all isolates have shown 100% resistance to the following antibiotics: FEP-cephalpim, CXM-Cefuroxime, CRO-Ceftriaxone, CAZ-Ceftazidim, CTX-Cefotaxime, ATM-Astreonam and AMP-Ampicillin. Such high resistance to cephalosporins is not common, but has been described by some researchers, for example, with 42.9% of CAZ-ceftazidim observed by Lei Tian and a group of researchers (Tian)...2018). It should also be noted that the vast majority of the above antibiotics are beta-lactam antibiotics in the broad spectrum and the reason for their resistance is the ability of *E.coli* to produce beta-lactamase in the extended spectrum of ESBL-extended spectrum. It is known that the mastic gene for beta lactamase is located in the plasmid and easily spreads. A new class of beta-lactam antibiotic monobactamate ATM-aztreonam has been found to be ineffective. In the case of carbopenems, 85% of isolates against IPM and MEM-meropenem have maintained sensitivity. Almost similar efficacy against these antibiotics *E.coli* has been found by various authors (imipenem - 93%) (Ullah ... 2009) (Meropenem - 98%) (Cambrea... 2015). Such a difference in antibiotic-sensitive research may be due to a different resistance mechanism.

ESBL-extended spectrum beta-lactamase masseur *E.coli* is characterized by associated resistance to other antibiotics. For example, gentamicin accounts for 80% to 40–60% of ciprofloxacin (Hoban...2011).

As a result of our study, CIP-ciprofloxacin had a resistance of up to 70-80% in *E.coli* isolates, with the same rate of LVX-levofloxacin, AMC-amoxacillin, DOX-doxycycline, and SXT-trimethoprim/sulfamoxazole. 74.6% resistance to ciprofloxacin has been described by cyber and co-authors (Kibret&Abera)... 2011), which is close to our results. From aminoglycosides - AMK-amikacin was relatively effective and 65% of isolants were sensitive to it, and in the case of gentamicin only 35%.

Based on our research, it can be said that only four antibiotics can be used as efficiently as possible against *E.coli,* such as CST-colistin (almost 100% susceptibility), PIP/TZP-piperacillin/tazobactam (88%) IPM and MEM-meropinem (85%) (diagram 7).

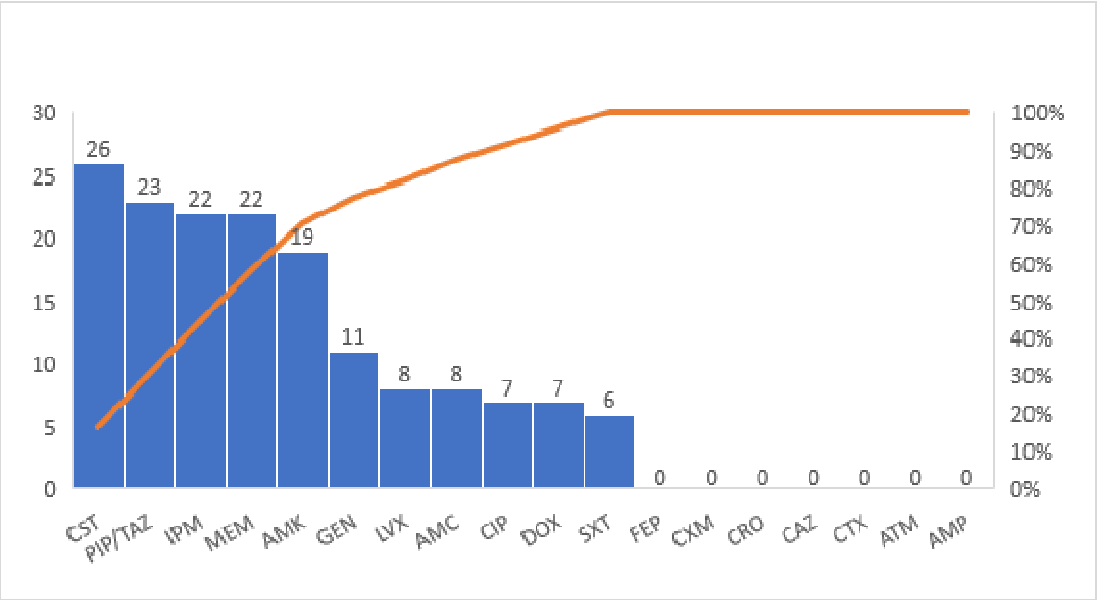


Diagram 7. Antibiotic sensitivity of E.coli separated from the samples

Such a high rate of antibiotic resistance is due to a number of reasons, namely: not all medical facilities have a microbiological diagnostic laboratory, so it is often necessary to apply to the laboratory of the Adjara region of one local disease control center to determine antibiotic sensitivity. The antibiotic-sensitive test itself takes 48-72 hours for incubation, so this is another extra time that often does not have a patient placed in the resuscitation department. All of this makes it forced the doctor to use a wide range of antibiotics, often completely ineffective.

The next reason is the unintentional use of antibiotics in outpatient practice, and in some cases it is attached to it, incorrect selection of antibiotics by a doctor and incorrect dose, or even the use of large amounts of generics in the pharmaceutical market, the quality of which is almost impossible to control.

Antibiotics are also used arbitrarily and uncontrollably by patients. Unfortunately, it is often impossible to control this because the purchase of atibiotics in the pharmacy network takes place without any prescription, even though the law on health care was amended in 2015 on the need for a prescription, but unfortunately failed to enforce the law effectively.

Another major problem is the use of antibiotics in livestock, poultry and fisheries, which then reach the human body with a chain of food products.

All of the above can lead to the rapid proliferation and spread of resistant strains among the population not only in our region but also beyond its borders, as Adjara is a resort area visited by a lot of tourists from different countries.

Results of molecular research of *E.coli* ESBL reproductive strains

It is impossible to determine the exact causes of antibiotic resistance without identifying genetic determinants. Only molecular-genetic studies that allow for the detection of mutations in the bacterial genome and the exact detection of genes responsible for resistance allow for phenotypic studies that allow for the determination of a reliable image. Therefore, at the next stage of the study, we performed a molecular analysis of the resistant strains detected by microbiological and biochemical methods.

In order to detect and specify resistance-encoding genes, samples were analyzed using methods proven in clinical microbiology: PCR (RAPID-PCR and multiplex PCR) and reverse hybridization. It has been established that *E.coli* is mainly the cause of ESBL resistance to pathogenic strains as a result of point mutations in TEM and SHVtype genes.

In recent times, however, there has been a sharp increase in resistance to CTX-M family genes (Yusha'u....2015; Hassuna....2020) We have determined the range of resistance for ESBL-generating resistant strains, by molecular-genetic analysis we have identified and identified CTX, IMP, VIM, OXA-18, NDM, KPS, TEM and SHV genes. The analysis of the multiplex PCR method allowed us to simultaneously obtain amplicons of genes of interest to us. Couples of specific primers R (revers) F (forward) were used for these genes (Table 10).

Table 10 Primers used for molecular identification of E.coli

gene	sequencing(5'- 3')
***bla*CTX-M**	5′- AAAAATCACTGCGCCAGTTC-3′
	5 ′- AGCTTATTCATCGCCACGTT-3 ′
***bla*IMP**	5' -GGT TTA AYA AAA CAA CCA CC- 3'
	5′- GGA ATA GAG TGG CTT AAY TC-3′
blaVIM	*5′-GATGGTGTTTGGTCGCATA- 3′*

	5´-CGAATGCGCAGCACCAG-3´
***bla*$_{OXA-48}$**	5' -GCGTGGTTAAGGATGAACAC- 3'
	5' -CATCAAGTTCAACCCAACCG- 3'
***bla*$_{NDM}$**	5´-GGTTTGGCGATCTGGTTTTG-3´
	5´-CGGAATGGCTCATCACGATC-3´
***bla*$_{KPC}$**	5' -CGTCTAGTTCTGCTGTCTTG- 3'
	5' -CTTGTCATCCTTGTTAGGCG- 3'
***bla*$_{TEM}$ F**	AAACGCTGGTGAAAGTA
***bla*$_{TEM}$R**	TAATCAGTGAGGCACCTATCTC
***bla*$_{SHV}$ F**	TTATCTCCCTGTTAGCCACC
***bla*$_{SHV}$R**	TGCTTTGTTATTCGGGCCAA

Gel-electrophoresis and reversible hybridization were performed to detect genes. The use of alternative approaches to the visualization of amplicons is not only for resistance-encoding genes, Aramaic

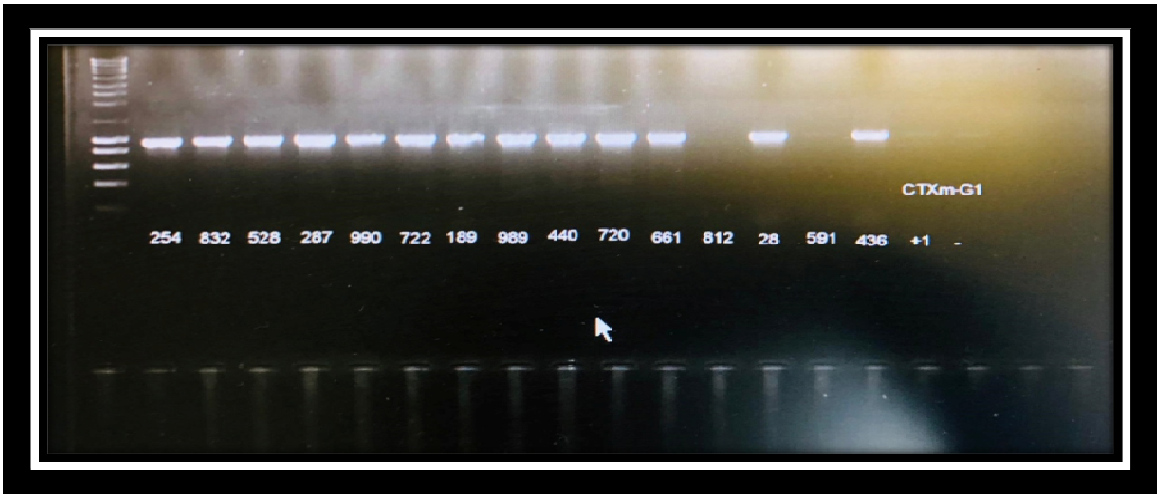
It allowed us to detect point mutations in genes.

In order to detect 15 strains of CTX, IMP, VIM, OXA-18, NDM, KPC genes selected from our research bank of *E coli*-resistant crops, gel-electrophoresis was performed in a 1.5% agarose gel. The CTX, KPC, TEM and SHV genes of the selected 7 strains were tested for reverse hybridization. Genes: CTX and KPC amplicons were tested by both methods to determine the validation of the experiment. We were particularly concerned with the CTX gene, which was due to the recent increase in the frequency of spread of this gene between the nosocomial strains of *E. coli* (Canton) ...2006).

To identify CTX genes and genotype phenotypic correlations, we conducted a PCR study in the clinical isolates of Escherichia coli, a derivative of the expanded spectrum beta lactamase. It is noteworthy that more than 172 variants of the CTX gene are known at this time, and six different groups of the CTX-M type are known. Despite the many variants of CTX genes, the most widespread is still the CTX-M-G1 group. CTX-M-G1 has been identified in isolates derived from enterobacteria in different regions of the world (Zhao...2013).

The results of the molecular analysis of the CTX-M-G1 gene conducted by us are presented on an electropherogram (pic.19). The length of the gene matching fragment is

864 bp. In gene amplification products, this gene was detected in 13 strains. Although all samples presented on the electropherogram are phenotypically resistant strains, in two cases no CTX-gene was detected.



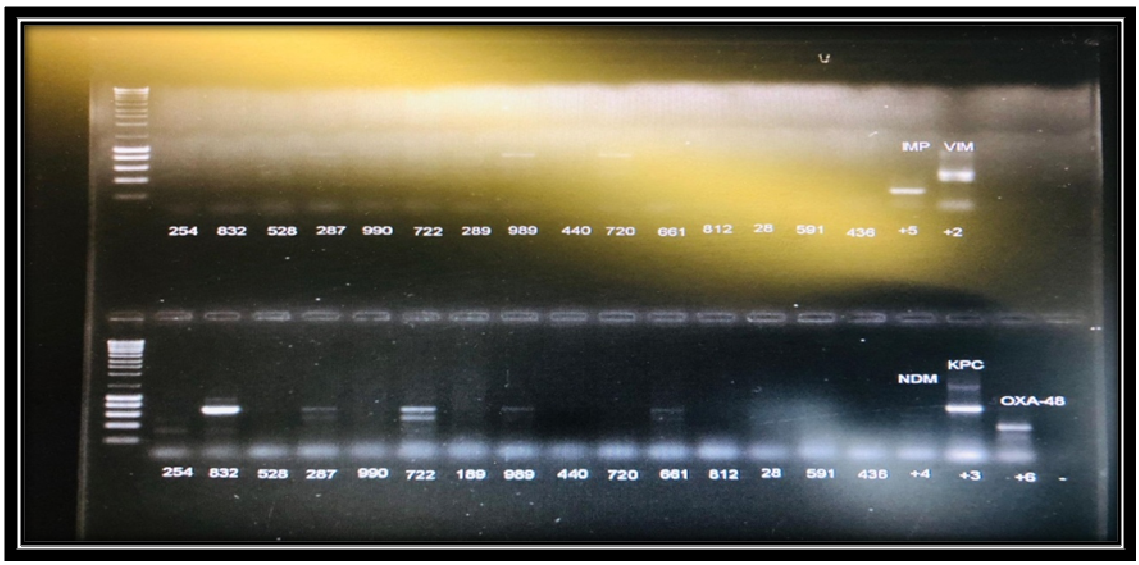
Pic 19 E.coli- PCR CTX-M-G1 gene

Carbopenamate-coaginating genes play an important role in the formation of bacterial antibiotic resistance to carbopenes. However, the synthesis of carbopenamases in *E.coli* strains is not as intense as in other microorganisms, although recent studies have shown that carbapenemresistant genes have been observed in *E.coli* isolates as well. It is thought that very soon he may acquire clinical significance (Galdino da Cruz Silva)...2020).

After determining the profile of antimicrobial resistance in the four samples studied by *E. coli* carbopene resistance was observed. So many, these patterns proved interesting in terms of molecular research.

Accordingly, we examined the samples examined on the CTX gene for sulfur-containing - OXA-18,KPC genes and metalobetalactamases - NDM, IMP, VIM genes.

The electrophoregram shows that IMP, VIM, OXA-18, NDM genes are not detected in any phenotypically resistant strain, while the KPC gene was observed in two (Pic. 20), The length of the fragment is 753 bp.



Pic. 20.E.coli on PCR OXA and KPC NDM MP VM genes

Table 11. The result of the molecular identification of

samples	PCR - CTX-M	PCR-GARB
254	CTXm	-
832	CTXm	KPC
528	CTXm	
287	CTXm	-
990	CTXm	-
722	CTXm	KPC
289	CTXm	-
989	CTXm	-
440	CTXm	-
720	CTXm	-
661	CTXm	-
812	-	-
28	CTXm	-
591	-	-
438	CTXm	-

Analysis of the results of the study showed that the results of phenotypic and molecular-genetic studies of antibiotic resistance are not always in full compliance.This can be

explained by the fact that the sensitivity of phenotypic analysis to mutations is relatively low. Nevertheless, in some cases, these methods of research are actually based on an identical result, which was well identified based on the study of the CTX-M gene in resistant strains of *E. coli*. Also, towards carbopinem we identified as phenotypic identified strains two of them was in relation to carbopens have been confirmed by positive molecular analysis on KPC (Table 11). Thus, these two methods really complement each other, and a complex approach increases the validity of research results. Detection of carbopemenamases in nosocomial specimens is an alarming signal also due to the tourist significance of the Adjara region, as these genes are easily transmitted through plazid and transposons and may be the cause of the formation and spread of epidemiologically endangered polyresist strains.

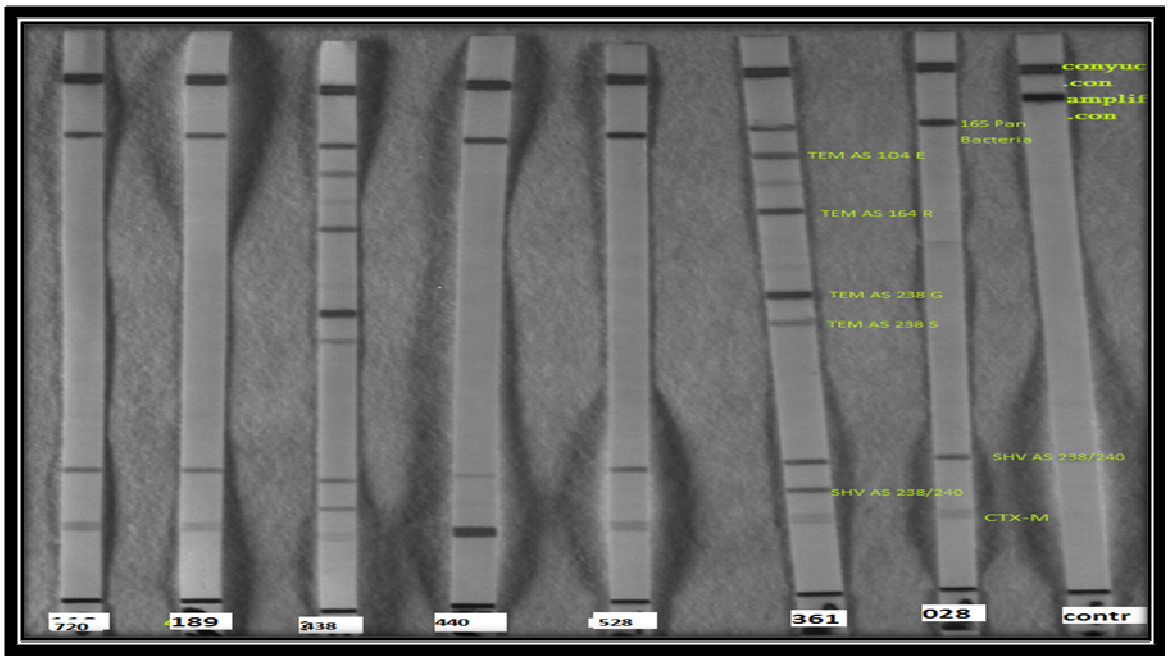
In addition, we selected seven samples from the resistant strains identified by us that showed resistance to penicillins, 3rd and 4th generation cephalosporins, and inhibitors (clavulanic acid, tazobactam). We analyzed these samples with the reverse hybridization method. This method has allowed us to simultaneously identify various genetic determinants: CTX-M, TEM AS 104 E, TEM AS 164 R, TEM AS 238 G, TEMAS 238 S, SHV AS 238/240 and KPC. Also, perform a detonation of mutant forms of beta-lactamase type genes.

The study identified the most well-known representatives of beta-lactamases containing sulfur group - TEM-1 and SHV-1 genes. It is these betalactamase genes that are most commonly found in intestinal sticks and other enterobacteria. Mutations in TEM-type enzymes The amino acid changes in relatively limited positions occur, but in these positions, for example, glutamate / lysine 104 position, arginine / serine / histidine 164 position, glycine / serine 238 position, and glutamate / lysine 240 position. It is thought that the formation of mutational variants is the result of selection of fluctive changes, and not beta-lactamases of one particular agent selection (Rahman...2018) are the most effective hydrolysis of penicillins. Enzymatic mutants are included in the extended spectrum group of betalactamase, which can effectively decompose not only penicillins but also cephalosporins of I-IV generations. It should be noted that the residues of serine in position 238 in beta-lactamases of the extended range of TEM type are crucial for the effective hydrolysis of ceftazidim, while lysine residues are important for the effective hydrolysis of cefotaxime.

To determine resistance mechanisms, TEM-type beta-lactamases are particularly interesting because their decoding genes are the result of the longest evolution and most often undergo mutations (Gregory)... 2017). SHV-1 genes, unlike TEM-type beta-lactamase genes, are less susceptible to change. Nevertheless, SHV-1 type beta-lactamases are no less interesting because resistance mechanisms are based on genetic characteristics. The genes encoding of this enzyme are localized in both bacterial chromosomes and plasids (Stepanova ...2011).

Pic.21 shows multiple PCR ampflicons on nitrocellular membranes in the form of lines that are localized to different positions.

Based on the analysis of the results obtained, we can conclude that the resistance of research strains was due to the presence of several genetic determinants simultaneously (Tabl 12). Both a wide range of beta-lactamase genes (wild type) and an enlarged spectrum (ESBL) have been identified that cause the beta-lactam ring to disintegrate and ultimately cause resistance to antibiotics.



Pic 21. Diagnosis of E.coli with multiple PCR amplification (Autoimmun Diagnostika Gmb HESBL)

As can be seen from the fifth table, mutations in the SHV AS 238/240 type and CTX-M gene were observed in all study strains. (G238^S/A and E240^>K) Amino acid substitutions cause an extension of the substrate specificity spectrum to cephalosporins, especially ceftazimid.

Table 12. Resistant genes detected by E.coli with multiple PCR amplification

ნიმუშები	PCR-TEM	PCR- SHV	PCR- *CTX-M- G1*	PCR*KPS*
720	-	*SHVAS 238/240 wild*	*CTX-M-G1*	-
189	-	*SHVAS 238/240 wild*	*CTX-M-G1*	-
438	*TEMAS 104 E wild* *TEMAS 164R wild* *TEMAS 238G wild* *TEMAS 238S ESBL*	*SHVAS 238/240 wild* *SHVAS 238/240ESBL*	*CTX-M-G1*	-
440	-	*SHVAS 238/240 wild*	*CTX-M-G1*	--
528	-	*SHVAS 238/240 wild*	*CTX-M-G1*	-
361	*TEMAS 104 E wild* *TEMAS 164R wild* *TEMAS 238G wild* *TEMAS 238S ESBL*	*SHVAS 238/240 wild* *SHVAS 238/240ESBL*	*CTX-M-G1*	-
258	-	*SHVAS 238/240 wild*	*CTX-M-G1*	-

Several determinants were identified in two of the study strains : CTX-M, TEM AS 104 E, TEM AS 164 R, TEM AS 238 G, TEMAS 238 Sda SHV AS 238/240. Mutant Forms of EBLS Class TEC Genes: EM AS 104 E, TEM AS 238 G, TEM AS 238 S conducts point mutations in positions 104, 164 and 238, respectively, here we would like to highlight the TEMAS 238 S genetic determinant identified in our study specimens. Gly23. The enzyme encoded by this gene is equally affected and easily removes cefotaxim and ceftazidim, while the beta-lactamase enzyme produced by the second sulfur group TEM AS 164 R gene (mutation Arg164Ser) is much more active in ceftazidine than in cefotaxime.

Thus, in the resistant strains of E. coli identified by different biological materials, a wide range of antibiotic resistance genes were identified: CTX-M, TEM, SHV, and in two samples, mutant forms were observed in combination with wild genes. It should be noted that these strains were removed from the runoff samples of medical catheters in patients with suspected catheters.

It is known that the whole range of microorganisms in the apk produced on the catheter can be, including gram-negative bacteria. These bacteria easily transmit genetic information to each other, including resistance genes.

CTX-M-G1 and SHV genes were detected in all seven strains using the multiplex PCR method. Therefore, we can assume that the resistance of intestinal sticks in the Adjara region

is determined by these genes. In one of the analyzed strains, which was phenotypically resistant to carbopens, resistance-encoding genes were not observed, as in gel-electrophoresis. This once again suggests that each clinically suspicious case requires a scurpulous approach and should be verified by molecular-diagnostic methods if necessary.

Conclusions :

1.*E.coli* intestinal sticks in the hospital sector of Adjara among the causes of nosocomial infection, 21% of the clinical isolates of gram-negative bacteria, and resistant to 12% ;

2.Antibiotics: FEP-cephalus, CXM-Cefuroxime, CRO-Ceftriaxone, CAZ-Ceftazidim, CTX-Cefotaxime, ATM-Aztreonam, and AMP-ampicillin are ineffective in the pathogenic strains of *E.coli;*

3. The most effective reserve antibiotic colistine and piperacillin / tazobactam are most effective against *E.coli* nosocomial strains; Amikacin and carbopenems are relatively less effective;

4. 88% of *E.coli*-resistant strains distributed in the region are reproduced by ESBL, which reduces the effectiveness of the use of cephalosporins against them;

5. Penicillins, Resistance to phenotypic-resistant strains with respect to cephalosporins and inhibitors of the 3rd and 4th generation was confirmed by CTX, By identifying TEM and SHV genes, And in two samples, along with wild genes, mutant genes of the ESBL class TEM

type were observed: TEM AS 104 E, TEM AS 238G, TEM AS 238 S; Identifying these genes in nosocomial strains minimizes the range of antibiotics used for treatment, Which in turn significantly complicates patient treatment, It increases the cost of delays in the hospital and can often be the cause of lethal end;

6. It is true that the OXA gene, which is responsible for the synthesis of carbapenem has not been identified, but the KPC gene has been observed, which to some extent explains only 85% of the sensitivity of carbapenems ; The appearance of strains of *E.coli* resistant to carbapenems is a disturbing signal, which confirms the undesirable global trend of widespread resistance to carbopenem;

7.Isolants of *E.coli* in Adjara do not reproduce metal-β-lactamases because they have not been identified in the reproductive IMP, VIM, NDM gene

Recommendations :

1. It is desirable to conduct trainings for physicians in the field of clinical microbiology, especially bacteriology, on the reasons for the rapid formation and spread of resistant strains;

2. Opening of microbiological laboratories in multifunctional hospitals, which will allow us to determine the cause of infectious disease in a timely manner and determine susceptibility to antibiotics; This will allow the doctor to change the medication in a timely manner taking into account the type and susceptibility of the isolated pathogen.

3. It is necessary to create a resistance profile to the database of circulating isolants in the hospital and their antibiotics, which will allow a list of leading susceptible antibiotics to be prescribed for the treatment of patients suspected of nosocomial infection. Targeted use of etiotropic, already known medications in treatment will reduce patient recovery and hospitalization. All this, in turn, will significantly reduce the cost of the patient's services;

4. It is advisable to examine all patients admitted to intensive care and resuscitation department, regardless of the source of the infection, in order to determine the presence of pathogenic microflora and the antibiotic resistance profile. This will allow us to establish a microflora from an early stage and avoid switching from the external population of nosocomial strains to the hospitals of the region ;

Works published around the dissertation :

An article to add

1. **V.Tavadze**, L. Akhvlediani1,2, R. Khukhunaishvili1, T. koiava1. ANTIMICROBIAL DRUG RESISTANCE OF ESCHERICHIA COLI WHICH CAUSE NOSOCOMIAL INFECTION

2.**V. Tavadze1**, L. Akhvlediani1,2, R. Khukhunaishvili1, T. koiava1, M. Nagervadze1. Antibiotic Resistance Status of E.coli Isolated from Intensive Care Units of Adjara Hospitals

3.**V. Tavadze1**, L. Akhvlediani1,2, R. Khukhunaishvili1, T. Koiava1. Genetic properties of blaCTX-M, blaSHV and blaTEM genes in ESBL producingE. coli clinical isolates

4.Koiava T1 , Gonçalves D2,3, Palmeira J2 , Arobelidze K.4 , **Tavadze V** 4 , Tediashvili M5 and Akhvlediani L.1 Ferreira H2 ,,MULTIDRUGRESISTANT ACINETOBACTER

BAUMANNII IN ADJARA REGION" Article DOI: 10.21474/IJAR01/xxx DOI URL: http://dx.doi.org/10.21474/IJAR01/xxx ISSN:2320-5407

5.Farlow J*c,d, Nozadze Ma, Mitaishvili Na, Kotorashvili Ae, Kotaria Ne, Arobelidze Ke, **Tavadze Ve**, Simsive Te, Imnadze Pe, Latif Na, Nikolich Mb, Washington Ma, Hinkle MKb, Kwon Pa, and Trapaidze NaComparative genomic analysis of four multidrug resistant strains of Acinetobacter baumannii from the country of Georgia. **November** 2019Journal of Global Antimicrobial Resistance 21.DOI: 10.1016/j.jgar.2019.11.002.

International Conferences:

1.The 8th International Bacillus ACT Conference will be held October 1-5, 2017 in Victoria, British Columbia.Canada. ANTIBIOTIC RESISTANCE OF BACILLUS CEREUS ISOLATED FROM NOSOCOMIAL INFECTIONS (BATUMI GEORGIA)

2.11 th International Congress for Veterinary Virology-ESVV-EPIZONE 2018, Vienna27-30 August, 2018. Human Anthrax Meningitis in the Black Sea Coast, Country of Georgia.

3. Randomized, Double-Blind, Multi-Center Study to Evaluate the Efficacy and Safety of Intravenous to Oral Solithromycin (CEM-101) Compared to Intravenous to Oral Moxifloxacin in the Creatment of Adult Patients with Community-Acquired Bacter.

4.Training-Central Asia-Eastern European Monitoring on Antimicrobial Resistance (CAESAR) Interpretations of Mycenae Determination Developed by the European

Committee for the Testing of Antimicrobial Sensitivity (EUCAST).Tbilisi.Lugarcenter.Figs.Ketorobelidze, nosocomial infections and antibiotic-reisistance profile

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