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RESEARCH ARTICLE

ANTIBIOTIC RESISTANCE STATUS OF *E.COLI* ISOLATED FROM INTENSIVE CARE UNITS OF ADJARA HOSPITALS

V. Tavadze*[1], T. Koiava[1], M. Nagervadze[1], R. Khukhunaishvili[1] and L. Akhvlediani[1,2]

[1]Batumi Shota Rustaveli State University
[2]BAU International University, Batumi

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ABSTRACT

Background: Nosocomial infections are the group of infections that pose the greatest threat in terms of their cause- microorganisms, developing and spreading resistance to antibiotics (Keskin Seremet, *et al*., 2020). In addition, prolonged hospital stays, violation of sterilization and disinfection regulations in some cases, lack of professionalism, and shortage of knowledge among medical workers (Butsashvili *et al*., 2010) contribute to the spreading of these infections and the development of antibiotic resistance. *E. coli* is the leading infection in the urinary tract. This bacterium is the one that predominates in both nosocomial and community-acquired urinary tract infections (Alaniz *et al*., 2018) (Plate *et al*., 2019)**. Objective:** To identify *E. coli* in samples with suspected nosocomial infection, isolate pure culture, and determine antibiotic resistance in various biological fluids. **Materials and Methods:** The study covered the following biological material obtained from the patients who spent 48 hours or more in the intensive care unit (total of 540): sputum, urine, material from the surface of the tip of venous and urinary catheters. Various biological samples underwent a retrospective study. The bacteria were isolated and identified with the 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 sensitivity to antibiotics was determined by Kirby-Bauer diffusion and E-test. The double-disk method was applied to determine the producers of *E. coli* broad spectrum beta-lactamase (ESBL). **Result:** The total of 540 samples was analyzed, out of which 236 were rejected as defective and 82 were gram-positive bacteria, and only 45 *E. coli* isolates were obtained from the remaining 158 samples. Thereof, 26 were resistant, and 23 were susceptible to antibiotics. Out of 158 samples, 89 were taken from sputum, –47 from urine, biological fluids-exudates from the tip of venous and urinary catheters. The highest degree of *E. coli* was found in sputum (56%) and urine (29%). The analysis for antibiotic sensitivity in samples showed that the resistance rate of *E. coli* isolated in the intensive care units in Adjara was quite high. All isolates were 100% resistant to antibiotics such as CXM/Cefuroxime, CRO/Ceftriaxone, CAZ/Ceftazidime, CTX/Cefotaxime, and AMP/Ampicillin. The resistance rate of 70-80% to FEP/Cefepim, CIP/Ciprofloxacin, LVX/Levofloxacin, ATM/Aztreonam, AMC/Amoxicillin/ clavulanic acid, and DOX/Doxycycline was recorded in *E. coli* isolates. **Conclusion:** Only 3 antibiotics can be effective against Escherichia coli such as CST/Colistin, IPM/Imipenem, and MEM/Meropenem.

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INTRODUCTION

Nosocomial infections, i.e. the ones contracted in hospitals, fall into the group of infections that pose the greatest threat in terms of their cause, microorganisms, developing and spreading resistance to antibiotics, since average of 80% of hospitalized patients are prescribed at least one antibiotic (Keskin Seremet, *et al*., 2020). In addition, prolonged hospital stays, violation of sterilization and disinfection regulations in some cases, lack of professionalism, and shortage of knowledge among medical workers (Butsashvili *et al*., 2010) contribute to spreading of these infections and development of antibiotic resistance. Moreover, these infections are further accompanied by complications, and patients are unable to leave the clinic in due time. This leads to rise in costs for medical care, and the mortality rate in such patients is also high (Tchouaket Nguemeleu *et al*., 2020). The spectrum of nosocomial infections and antibiotic sensitivity varies by countries, regions, and sometimes hospitals. In the period between 2014 and 2016, China (Wuhan province) was dominated by *Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae.* According to the studies done in Turkey from 2010 to 1018 (KeskinSeremet *et al*., 2020), the

leading cause of nosocomial infection was a gram-negative bacteria: *Acinetobacter spp*. (30%), *Klebsiella spp*. (15.2%), *Pseudomonas spp.* (14.4%), and *Escherichia coli* (4.2%). The studies performed in Georgia (the capital: Tbilisi) between 2007 and 2010 indicate that the leading microorganisms among nosocomial infections were as follows: *Klebsiella pneumoniae (26.5%); Pseudomonas aeruginosa (15.2%); Candida albicans (12.3%); Staphylococcus aureus (9.0%); Escherichia coli (7.6%),* and *Acinetobacter baumannii (5,1%)* (Kandelaki *et al*., 2011); and the study *Identification of Acinetobacter Spp. As Determination of Hospital Infection Causes and Antibiotic Resistance Profile, n.d.* done in Batumi (Adjara region) from 2014 to 2017, Show the prevalence of the following bacteria: (PDF), *Acinetobacter, Escherichia coli, Pseudomonas aeruginosa*, and *Klebsiella pneumonia.* The research in Adjara studied the mechanisms causing resistance in these bacteria with the exception of Escherichia coli, have been studied.

E. coli is the most common faceted intestinal anaerobes and is typically a harmless gram-negative bacillus; however, in some cases it causes a number of serious diseases. This bacterium is most often associated with urinary tract infections and is often caused by various medical manipulations, namely: urinary catheterization (31%), surgical infection (17%), primary blood infection, particularly associated with the use of vascular catheters (14%), and artificial ventilation induced lung pneumonia (13%) (Tulear, *et al*., 2018). *E. coli* is the leading infection in urinary tract, followed by Proteus spp., *Staphylococcus saprophyticus*, *Klebsiella spp.* and other *enterobacteriaceae.* This bacterium is the one that predominates in both nosocomial and community-acquired urinary tract infections (Alaniz *et al*., 2018) (Plate *et al*., 2019). Since *E. coli* is one of the microbes that cause nosocomial infections in the region, and its sensitivity to antibiotics has not been studied, the purpose of our study was to identify *E. coli* in the biological fluids of the patients suspected to have nosocomial infection, and to determine the sensitivity to antibiotics.

MATERIALS AND METHODS

The material for the study was the following biological material from patients who had spent 48 hours or more in the intensive care unit and have the respiratory, intra-abdominal, skin and soft tissues, and urinary tract infections: sputum, urine, biological fluids and material from the tip of the venous and urinary catheters. The total of 540 samples were obtained and examined.

Samples and microscopic examination: The samples were processed and inoculated in digest medium in accordance with the biosafety and quality control regulations. The matter was taken in two samples. After 24-28-hour incubation period, with bacteria being in place, a Gram-stained smear was prepared for initial identification of the microbe. Meanwhile, all samples were plated on the following digest medium: sheep blood agar of 5%, MacConkey agar, endo agar, and CHROMagar. The inoculation by streaking methods was applied to obtain pure culture. For quantitative determination, inoculation was performed with a calibrated loop of 0.01 μL.
The seeded plates were placed in a thermostat set at 36° C, and after 24 hours of incubation the plates were checked. Colonies morphologically resembling *E. coli* were selected, namely: high lactose-positive colonies with metallic luster on the Endo digest medium zone; colonies with positive / negative lactose on the MacConkey digest medium -; large, gray, gamma or beta hemolytic colonies on the 5% of the sheep digest medium; large characteristic brown colonies on the area of chromium agar medium. The matter was again Gram tainted. After microscopy, all gram-negative bacilli were plated on Soybean-Casein digest medium to obtain a subculture. After 24 hours of incubation, the inoculated plates were checked.

Biochemical method: Primary biochemical tests were performed on subcultures obtained from Soybean-Casein Digest. Medium. Based on the results from the primary biochemical tests, the final identification of all suspected cultures was performed with the application of the API 20 E Identification System. The suspension was prepared from the 24 hour subculture according to the McFarland 0.5 turbidity standard. The reactions were recorded with the use of the interpretive table. The resulting seven-digit code was identified with the identification table and the analytical catalog /https://apiweb.biomerieux.com.

Antibiotic Susceptibility Test: Antibiotic susceptibility test was performed using the Kirby-Bauer method on Mueller-Hinton agar in accordance with EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute) guidelines and using 19 antibacterial agents (table 1). The E. coli isolates were inoculated in normal saline solution to a density of 0.5 McFarland turbidity standard. Cotton swabs were used for streaking the diluted onto Mueller-Hinton agar plates. The antibiotic discs were placed 30 mm apart and 10 mm away from the edge of the plate. The plates were inverted and incubated aerobically at 35°C for 18 to 20 hours. The zone of inhibition and resistance was measured, recorded, and interpreted (S-susceptible, I-Intermediate, R-resistant) according to the recommendation of the EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute) guidelines. The ATCC strain of *E. coli* 25922 was used as a control strain. All isolates were tested for susceptibility to the antibiotics listed in the table below (Table 1).

Table 1. List and of antibiotics and acronyms

N	Acronyms	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

Sensitivity quality control (ATCC) was performed with *E. coli* ATCC 25922® and *E. coli* ATCC 35218®, the reference strains of typical cultures from the American collection.

Colistin Sensitivity Electronic Test: Test strip E with a gradient of various antibiotic concentrations was placed on the surface of the dense digest medium with inoculated strain under study. After 18–24 hour incubation period, an oval growth retardation zone would form. The result (the value of the minimal zone of inhibition) was determined at the intersection point between the strip and the zone.

Extended Spectrum β-Lactamase (ESBL) screening and Double Disc Synergy (DDST) confirmatory tests: For the preparation of inoculonum was used 24 hours subculture. In the initial screening test, a disc of amoxicillin + clavulanic acid (20 + 10 µg) was placed in centre of the Petri plate already inoculated with the test organism while aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) discs were placed within the distance of 20 to 30 mm (center to center) from the amoxicillin + clavulanic acid disc on the same plate. The zones of inhibition around the third-generation cephalosporin discs and aztreonam were observed after 18-20 hours of incubation at 35°C. If the zone of inhibition around one or more cephalosporin discs and aztreonam was extended to the side nearest to the amoxicillin + clavulanic acid, the organism showing this synergy was labelled as ESBL positive. In the phenotypic confirmatory test, the *E. coli* isolates were inoculated in normal saline solution to a density of 0.5 McFarland turbidity standard. Cotton swabs were used for streaking the diluted onto Mueller-Hinton agar plates and discs of cefotaxime (30 µg) and ceftazidime (30 µg) separately, and each of these in combination with clavulanic acid (10 µg) were placed on the surface of the lawn of bacteria. The difference of 5 mm between the zone of inhibition of a single disc and in combination with clavulanic acid was considered as ESBL positive isolate. For tests purposes, standard discs were used according to the recommendation of EUCAST and CLSI. The ESBL producer strain (ATCC) – *E. coli* ATCC 25922®was used as the control.

RESULTS AND DISCUSSIONS

The total of 540 samples was taken from patients in the intensive care units in Adjara, who stayed in hospital for at least 48 hours. Of the obtained samples, 236 did not meet the quality standard, and 82 samples contained gram-positive bacteria. The remaining 158 samples distributed as 89 found in sputum, 47 in urine, and 22 in biological fluid-exudate taken from the tip of the venous and the urinary catheters. E. coli was found in 48 samples out of the aforementioned 158. Of the 26 samples, 23 were identified as ISBL producers. The study showed that *E. coli* isolates were 100% resistant to the following antibiotics: FEP-cefepime, CXM/Cefuroxime, CRO/Ceftriaxone, CAZ/Ceftazidime, CTX/Cefotaxime, ATM/Aztreonam, and AMP/Ampicillin. 83% of urinary isolates were resistant to CIP/Ciprofloxacin, LVX/ Levofloxacin, and DOX/Doxycycline, 67% were resistant to AMC/Amoxicillin-clavulanic acid and SXT/Trimethoprim/ Sulfamethoxazole. Only one isolate was found to have intermediate sensitivity to GEN/Gentamicin. The highest sensitivity rate of *E. coli* isolates was observed to PIP/TZP/Piperacillin/tazobactam (83%), followed by 79.6% sensitivity to CIP/Ciprofloxacin, and 67% to AMK/Amikacin (Fig. 1). *E. coli* isolates from sputum were found to be highly resistant to 8 antibiotics. In particular, the 100% resistance to the following antibiotics was detected: FEP, CXM, CRO, CAZ, CTX, IPM, ATM, and AMP. The sensitivity recorded to CIP, LVX, AMK, AMC, SXT, DOX, and GEN was below 50%; whereas, the 100% sensitivity was observed in case of PIP/TZP and CST (Fig. 2).

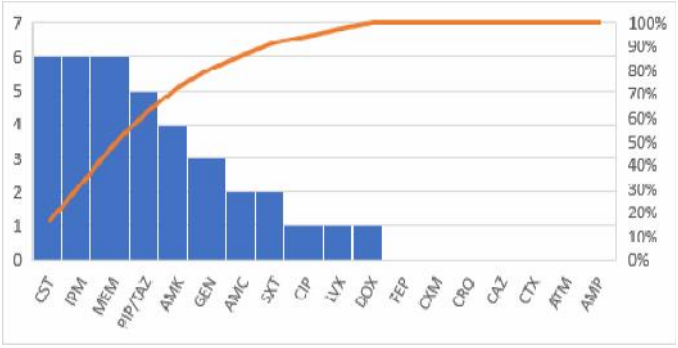

Figure 1. Antibiotic sensitivity of E. coli isolated from the urine samples

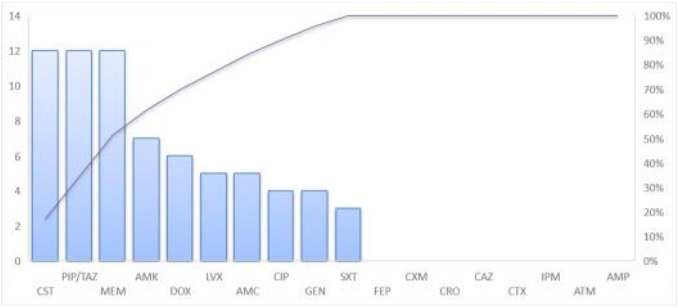

Figure 2. Antibiotic sensitivity of E. coli isolated from the sputum samples

The analysis of isolates obtained by flushing the venous and urinary catheters showed that *E. coli* is resistant to 7 antibiotics. In particular, the 100% resistance to the following antibiotics was detected: FEP, CXM, CRO, CAZ, CTX, AMP, and DOX. The 80% resistance was observed against CIP, LVX, AMC, and SXT. No Intermediate sensitivity was detected to either of the antibiotics, and the 100% sensitivity was maintained by *E. coli* to only 4 antibiotics: CST, IPM, AMK, and MEM (Fig. 3).

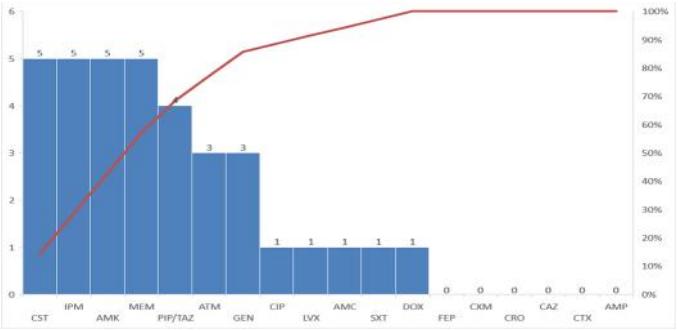

Figure 3. Antibiotic sensitivity of E. coli isolated from samples flushed off the catheters

Thus, the resistance of *E. coli* isolated in the intensive care units in Adjara was quite high. If all types of samples are taken together, all isolates showed 100% resistance to the following antibiotics: CXM/Cefuroxime, CRO/Ceftriaxone, CAZ/Ceftazidime, CTX/Cefotaxime, and AMP/Ampicillin. Such high resistance to a large number of antibiotics is not a frequent case. For example, Lei Tian and the authors showed that the resistance of *E. coli* to CAZ/Ceftazidime is 42.9%

(Tian, *et al*., 2018). The resistance up to 70-80% was recorded for *E. coli* to FEP/Cefepime, CIP/Ciprofloxacin, LVX/Levofloxacin, ATM/Aztreonam, AMC/Amoxicillin-clavulanic acid, and DOX/Doxycycline isolates. . Similar resistance of 74.6% to Ciprofloxacin was reported by Kibert, *et al*. (* Kibret & Abera, 2011). Based on our research, it is possible to state that only 3 antibiotics can be effectively used against *E. coli*, such as CST/Colistin, IPM/Imipenem, and MEM/Meropenem (100%). Nearly the same sensitivity of 93% and 98% to Imipenem and Meropenem, respectively, were observed by different authors (Ullah *et al*., 2009) (Cambrea, 2015) (Fig. 4).

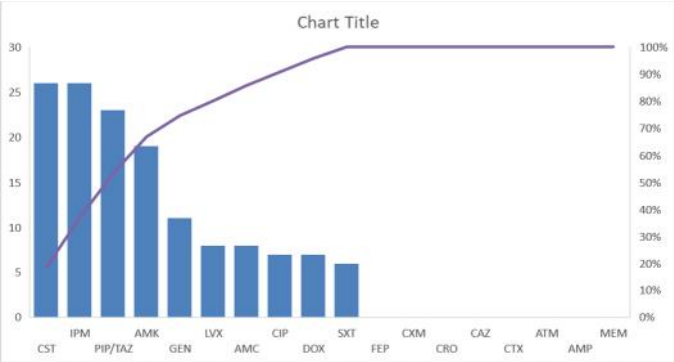

Figure 4. Antibiotic sensitivity of E. coli

Conclusion

This high rate of resistance to antibiotics is caused by to a number of reasons, in particular: excessive and uncontrolled use of antibiotics in the population, use of wide range of antibiotics in clinics, and complete disregard for antibiotic susceptibility (the frequent reason for this is that testing requires some time and leads to delay in giving antibiotics to patients). Such rapid growth and escalation of antibiotic resistance is the result of the rapid spread of resistance genes. The necessary measure is to identify the genes responsible for the resistance.

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