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THE RESISTANCE TO QUINOLONES OF SOME ENTEROBACTERIACEAE STRAINS ISOLATED FROM THE URINARY TRACT

Cristina PERDIVARĂ[1], Dumitra RĂDUCANU[1*], Daniela TIȚĂ[2], Diana-Elena MAFTEI[1], Ionuț STOICA[1]

[1]"Vasile Alecsandri" University of Bacau, Faculty of Sciences, Department of Biology, 157 Calea Mărăşeşti Street, 600115, Bacău, Romania, cristinapavel@yahoo.com, danielatita2007@yahoo.com, ionut_stoica23@yahoo.com
[2]County Emergency Hospital, 2-4 Spiru Haret Street, Bacău, Romania, danielatita2007@yahoo.com

KEYWORDS

Antibacterial resistance *Enterobacteriaceae* Urinary tract infections (UTIs) Quinolones

ABSTRACT

The pathogenic microorganisms related to the UTIs etiology frequently belong to the *Enterobacteriaceae* family. This present study aims the following: to determine the *Enterobacteriaceae* isolated from the urinary cultures, displaying the most common species, such as: *Escherichia coli*, *Klebsiella spp.*, and *Proteus spp*.; to test the biochemical and culture medium-related characteristics by means of some multi-test medium variants (TSI, MIU, Simmons Citrate) and of the API 20 E kit (Biomerieux) to identify the species; to correlate the microbiological data with the clinical factors. The survey on the distribution and number of UTIs from Moldavia region (Romania) during the analyzed period of time pointed out a rather good health state in the human population based on the pathogens' frequency and the resistance patterns.

INTRODUCTION

The urinary tract infections (UTIs) are some of the most common medical conditions, highly prevailing, yet unevenly distributed by gender, age, physiological and pathological health conditions. The pathogenic microorganisms related to the UTIs etiology frequently belong to the *Enterobacteriaceae* family (Buiuc et al., 2022; Mihai et al., 2019). They may develop complex pathogenic mechanisms, e.g. the synthesis of adhesins, enzymes and toxins, and the biofilm formation or the presence of some antigens that facilitate the invasion and survival inside the host (Sajid et al., 2025; Flores-Mireles et al., 2015; Partridge, 2015).
The UTIs are clinically assessed as severe or mild, and rarely interfere with healthy people. The risk factors linked to the mild UTIs are: predisposition in women, previous UTIs, sex life, vaginal infections, diabetes, obesity and genetic propensity (Storme et al., 2019; Buiuc et al., 2022).
The severe UTIs occur together with risk factors that are detrimental to the urinary tract. The factors that trigger these severe UTIs are: the mechanic alteration of the urinary flow by the kidney stones obstruction, prostate hypertrophy, ureter compression caused by an enlarged uterus in pregnant women, abdominal or pelvic tumor growth, urinary retension, kidney transplant, diabetes etc. (Foxman, 2014; Ahmed et al., 2023; Singh, 2020).
The Covid pandemic altered the pathogenic bacteria effects on some virally immunodepressed organisms (Chibabhai et al. 2020, Lai et al., 2021) One of these delicate conditions refers to the post Covid antimicrobial resistance to UTIs following the antibiotic treatment, that decreases the complications associated with the viral infections, yet bearing a high risk to human health (Silago et al., 2025). The antibiotics overdose and the sub-therapeutic intake for short periods of time require a reassessment of therapeutic strategies in the cure of the UTIs. This fact implies an accurate and fast microbiological diagnosis. The lab techniques and the test interpretation are essential to prevent bacterial resistance to antibiotics. Mareș et al. (2022) displayed several types of bacterial resistance for certain bacterial strains frequently related to the dynamics of the urinary infections. They were linked

* Corresponding authors: Răducanu D., Maftei D.
E-mail address: dora.raducanu@ub.ro, diana.maftei@ub.ro
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to the peculiarities of the pre- and post-Covid pandemics (particularly the strains liable to achieve bacterial multiresistance, such as: *E. coli*, *Pseudomonas spp*., *Klebsiella spp*., and *Enterococcus spp* (Mareș et al., 2022; Martins, 2023).
The pathogens must be continuously observed in case of the UTIs occurrence. Their sensitivity to antibiotics must be assessed, together with the various clinical factors (gender, age, comorbidities) along with the socio-economic factors (environment, life style etc.) (Hogea et al., 2024). These data are very important to all the specialists and physicians in the cure of the UTIs, because they may prevent the multiresistance and the lethal superbacteria.
This present study aims the following:

- to assess the enterobacteria isolated from the urinary cultures of the potential UTIs patients, emphasizing the most frequent species, such as: *Escherichia coli*, *Klebsiella spp*., and *Proteus spp.*
- to test the biochemical and culture medium-related characteristics by means of some multi-test medium variants (TSI, MIU, Simmons Citrate) and of the API 20 E kit (Biomerieux) to identify the species,
- to correlate the microbiological data with the clinical factors (age, gender, comorbidities).

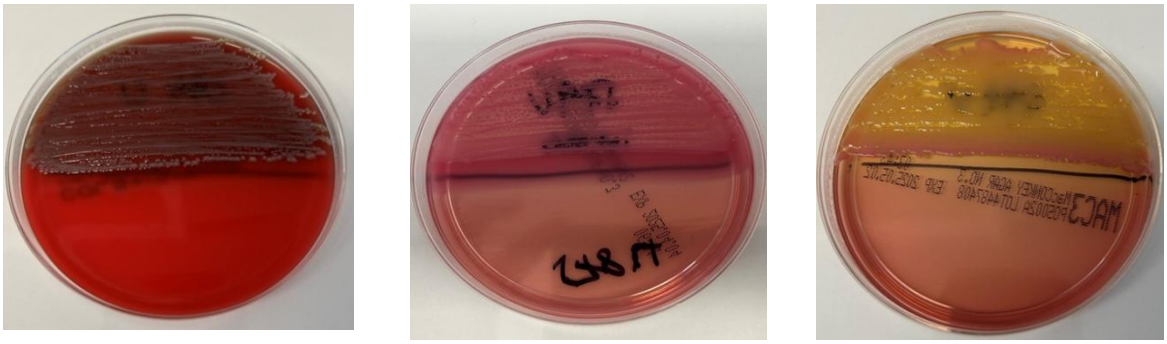
MATERIALS AND METHODS

The study was conducted during January 2023 - June 2024 on 948 patients from a laboratory in the Moldavian region (Romania). A total of 402 patients tested positive for UTIs caused by pathogens from the *Enterobacteriaceae* family. There was assessed the sensitivity to antibiotics (fluoroquinolones) of the isolated strains by means of the antibiograms. The lab technique was Kirby Bauer. The results were interpreted according to the international standards, CLSI (Clinic and Laboratory Standard Institute, USA), respectively.
The urine samples were at first biochemically analyzed, and subsequently the cultures were quantitatively assessed. The biochemical test of the urine samples consisted of the automatic analysis of the urine strips (Uricell 1280). The quantitative test was based on the calibrated inoculation loop method. The following steps were proceeded: the urine sample was mildly shaken to homogenise; the culture medium variants (blood agar and Mac Conkey) were inoculated with the urine sample (10µL/culture plate) in aseptic conditions. The method facilitated the growth of the isolated colonies, from which the bacterial strains will be identified and the antibiogram done. Several strains of bacteria (mainly Gram-positive) grow on the blood agar plate. The Mac Conkey selective medium points out the differences between the Gram-negative bacteria and impends the *Proteus* infections, that may occur on the blood agar culture medium. The inoculated plates are incubated from 18 to 24 hours at 37° C, and subsequently analyzed. The colonies belonging to each strain are numbered.
Therefore, the bacteriuria is calculated using the formula:

$$X = NxDx\frac{1}{volume\ of\ inoculated\ sample}$$

X = number of CFU/ml of urine
N = number of colonies/plate
D = dilution factor (reversed dilution)

Once the quantitative tests for urocultures were performed, the bacteriuria tests over 10^5 CFU/mL were analyzed, with a focus on the *Enterobacteriaceae*. The cultural properties for the bacterial colonies provided by the positive urocultures were: the colonies grow in 18 to 24 hours on basic nutritive medium culture at 35-37°C (Figure 1).



A B C

Figure 1. Positive uroculture – colonies specific to Gram-negative bacilli (≥ 10^6 UFC/mL) on: Columbia blood agar (A) – S-type colonies; lactose-positive Mac Conkey colonies (B) – S-type colonies, (C) – mucoid colonies (original photo)

They are aerobic/facultative anaerobic microbes. Most strains grow S-type colonies on agar medium. The colonies are round, domed, smooth, with rough edges, 1-3 mm in diameter. *Escherichia coli* caused haemolysis on ram blood agar. Among other peculiarities, there were noticed mucoid, round, bulging colonies tending to merge. Their diameter ranged between 3 to 4 mm in *Klebsiella* and *Escherichia*. An invasive growth characterized *Proteus*. The colony grew a vail covering the ram blood agar medium. The Mac Conkey low selective medium provided the growth of some lactose-positive colonies, pinkish-red, 2-4 mm in diameter, rarely mucoid or surrounded by an opalescent halo, and lactose-negative colonies, colourless, semi-transparent, 1-2 mm in diameter.
Primarily, the identification of the *Enterobacteriaceae* isolated from urocultures, based on some biochemical characteristics, was assessed on several multitest medium variants: TSI – Triple Sugar Iron Agar, MIU – Motility Indole Urea, Simmons Citrate agar. The lab work was in accordance to the sterile conditions required, and consisted of the collection (by means of a sterile inoculation loop) of a single colony provided by the primary culture, followed by a stinging inoculation of the MIU medium, continued with a zig-zag inoculation at the tilted surface of the TSI and Simmons Citrate agar medium within the test tubes.
After the inoculation on the MIU medium, a filter paper band containing the Kovacs reagent was inserted inside the tube, to evince the synthesis of indole. The tubes were incubated at 35-37^{o} C for 18 to 24 hours. The preliminary identification of the main pathogenic *Enterobacteriaceae* in urocultures based on some biochemical criteria is displayed in the table below:

Table 1. The primary identification of the *Enterobacteriaceae* isolated from urocultures, based on some biochemical characteristics (adapted after Buiuc, 2022 lab technique)

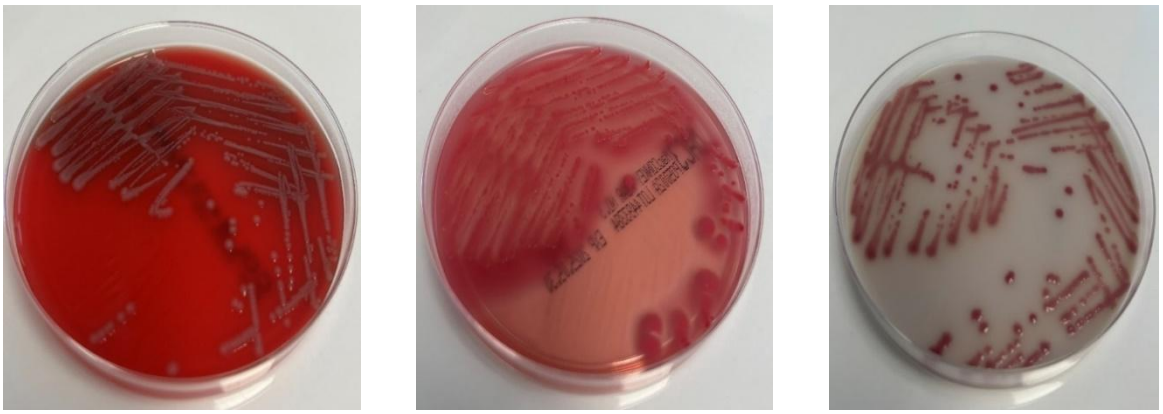
Genus	Triple Sugar Iron medium (TSI)				motility, indole, urea (MIU)			Simmons Citrate (SC)
	Glucose	Gas	H_2S	Lactose Saccharose	Motility	Indole	Urease	
Escherichia	+	+[a]	-	+a	+[a]	+[c]	-	-
Klebsiella	+	+[b]	-	+b	-	V	V	+[b]
Proteus	+	+	+	-	+	V	+	V
Enterobacter	+	+	-	+	+	-	V	+
Citrobacter	+	+	V	+	+	V	+/-	+
Serratia	+	V	-	V	+	-	-	+

Legend
„+" positive strains; „-"negative strains; „V" - variations related to species
[a] exception *E. coli* inactive
[b] exception *K. rhinoscleromatis*
[c] exception *E. vulneris*

This present study aiming the selective identification of the *Enterobacteriaceae* pathogens was performed with the API 20 E biochemical kit (Biomerieux). The inoculation and the interpretation of the strips were handmade. An APIWEB (Biomerieux) soft allowed the identification of the strains. The lab experimental method follows the API 20 E (Biomerieux) instruction kit. The results' interpretation form comprises the API 20 E tests divided into groups of three/each group. Every test was assessed by a digit (1, 2, or 4). By adding the corresponding numbers for each positive reaction within each group, a numeric profile (containing 7 digits for all the 20 tests related to the API 20 E strip) was provided. The identification of the isolated microorganism is accomplished by means of the numeric profile, along with the identification software APIWEB (following the API 20 E protocol kit, Biomerieux).

RESULTS AND DISCUSSION

Of all the analyzed urocultures (948), it was noticed that 71% of them tested negative.
The tests on the frequency and characteristics of the pathogens causing UTIs pointed out that most of them belong to the *Enterobacteriaceae* family (Table 1).
The identified cultural characteristics were:
The *Escherichia* genus was identified on basic culture medium deprived of inhibiting compounds and grew S-type colonies. On the blood agar culture medium, several strains grew haemolytic colonies. The Mac Conkey medium provided lactose-positive colonies. The UriSelect chromogenic medium grew pink colonies (Figure 2). The TSI culture medium produced gas, and H_2S was not released, the glucose was acidified and the reaction was lactose-positive. The MIU medium was characterized by motile colonies, most strains formed indole, and they are urease-negative. The Simmons-Citrate medium did not metabolize the citrate (Figure 3).



A B C

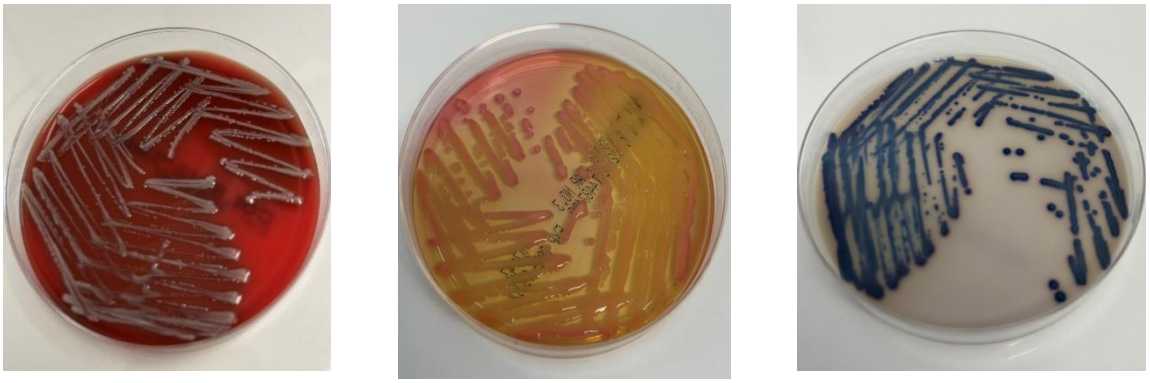
Figure 2. *E. coli* culture on: Columbia ram blood agar (A); Mac Conkey medium (B); UriSelect chromogenic medium (C) (original photo)

A .
TSI: G+, L+, Z+, H_2S-, gas+
MIU: motile, urease -, indole+
SC: -

B. *E. coli* identification by means of an API kit

Figure 3. The biochemical identification of *E. coli* on multitest medium variants: TSI: G+, L+, Z+, H_2S-, gas+, MIU: motile, urease -, indole+, SC: - (A) and identification by API kit E (B) (original photo)

The *Klebsiella* genus was identified by the following characteristics (Figures 4, 5):

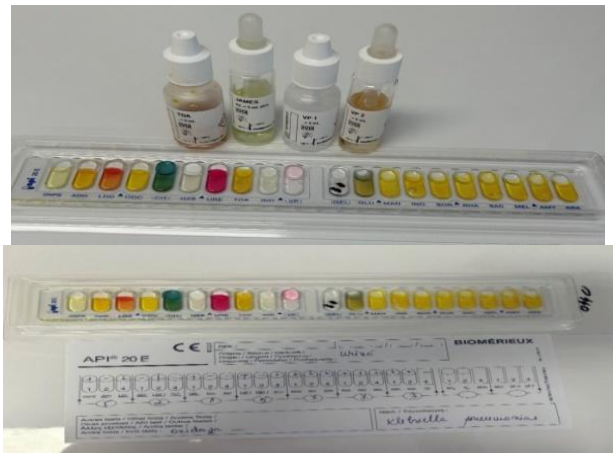


A B C

Figure 4. *Klebsiella pneumoniae* on: Columbia blood agar (A); Mac Conkey medium (B); UriSelect chromogenic medium (C) (original photo)

A

TSI: G+, L+, Z+, H_2S-, gas
MIU: motile, indole -, urease positive
SC: +



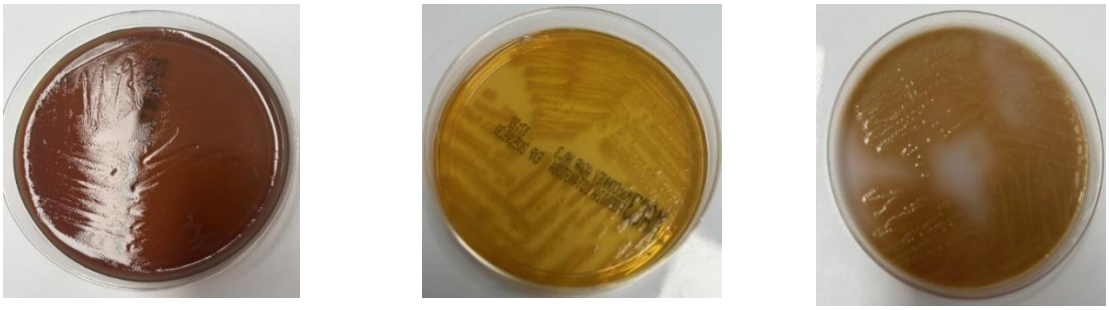
B

Identification of *K. pneumoniae* on an API 20 E kit - (A),(B) (original photo)

Figure 5. The biochemical identification of *K. pneumoniae* on some multitest culture medium variants: TSI: G+, L+, Z+, H_2S-, gas ,MIU: motile, indole -, urease positive , SC: + (A) and Identification of *K. pneumoniae* on an API 20 E kit (B) (original photo)

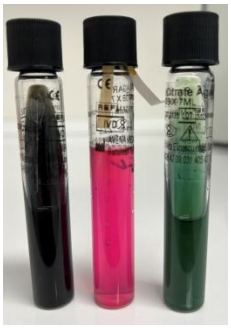
- It grew large colonies, round and bulging, mucous (they flow' on the medium surface), tending to merge; the Mac Conkey medium initially grew mucous lactose positive colonies. The colonies turned lactose-negative 24 hours later, due to the ‚chameleonic reaction' occurring in alkaline medium.
- The UriSelect chromogenic medium grew dark-blue colonies
- The TSI culture medium did not release gas, acidified the glucose, and it is lactose - saccharose positive
- The MIU medium – not motile; some species can provide indole, that differentiates *K. oxytoca* (indole-producing species) from *K. pneumoniae*; urease-positive
- The SC medium metabolized the citrate.

The *Proteus* genus identified in the analyzed urocultures was characterized by the following aspects (Figures 6, 7):



A B C

Figure 6. *Proteus vulgaris* culture on: Columbia blood agar (A); Mac Conkey medium (B); UriSelect chromogenic medium (C) (original photo)



A

TSI: : G+, L-, Z+, H2S+
MIU: motile, indole -, urease positive
SC: -

Figure 7. The biochemical identification of *P. vulgaris* on some multitest culture medium variants: TSI: : G+, L-, Z+, H2S+, MIU: motile, indole -, urease positive, SC: - (A) (original photo)

- The migration process occurred on some culture medium variants (Columbia blood agar); the concentric migration vails start from the inoculation point and reach the periphery of the plate or the borderline of another colony's vail; if the two vails belong to the same species, they merge into a continuous vail; if the colonies belong to different species, a line appear in between (the Dienes phenomenon)
- Lactose-negative bacteria
- They grow brown colonies on the UriSelect chromogenic medium
- On the TSI culture medium – they produce H_2S, acidify glucose; lactose-negative
- It is motile on the MIU medium; some species produced indole (e.g. *P. mirabilis*) and this makes the distinction from *P. vulgaris* (it does not produce indole)
- It is urease-positive
- Some species metabolize the citrate

The main etiologic agents depicted in the UTIs in the tested samples (673) during January 2023 – June 2024 were: *E. coli* in 207 samples, followed by *Proteus spp* in 16 samples and *Klebsiella spp* in 3 samples (Figure 8).

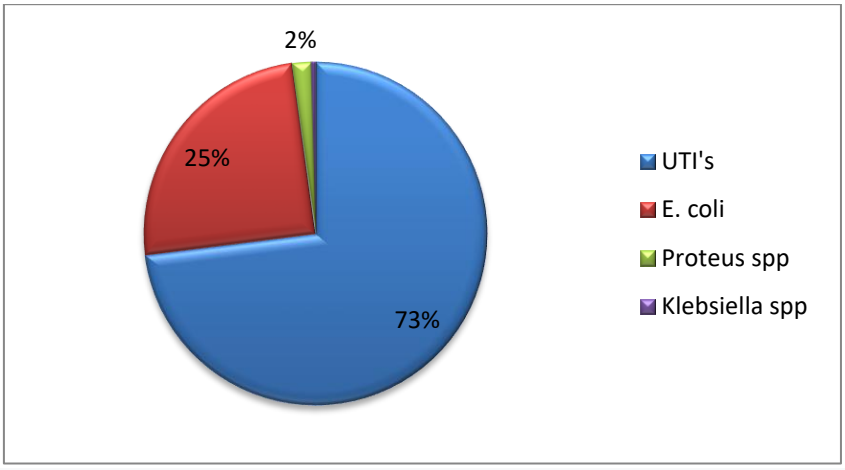


Figure 8. Percentage distribution of pathogenic bacteria in urinary tract infections

Escherichia coli was the main pathogenic agent that caused UTIs in both females (86%) and males (85%). The minor difference pointed out that *E. coli* is not gender-related.

The high incidence of the UTIs occurred in people over 46 years of age (both in females and in males). This distribution indicated a high occurrence of UTIs in elderly people, probably related to other comorbidities or to a weakened immune system.

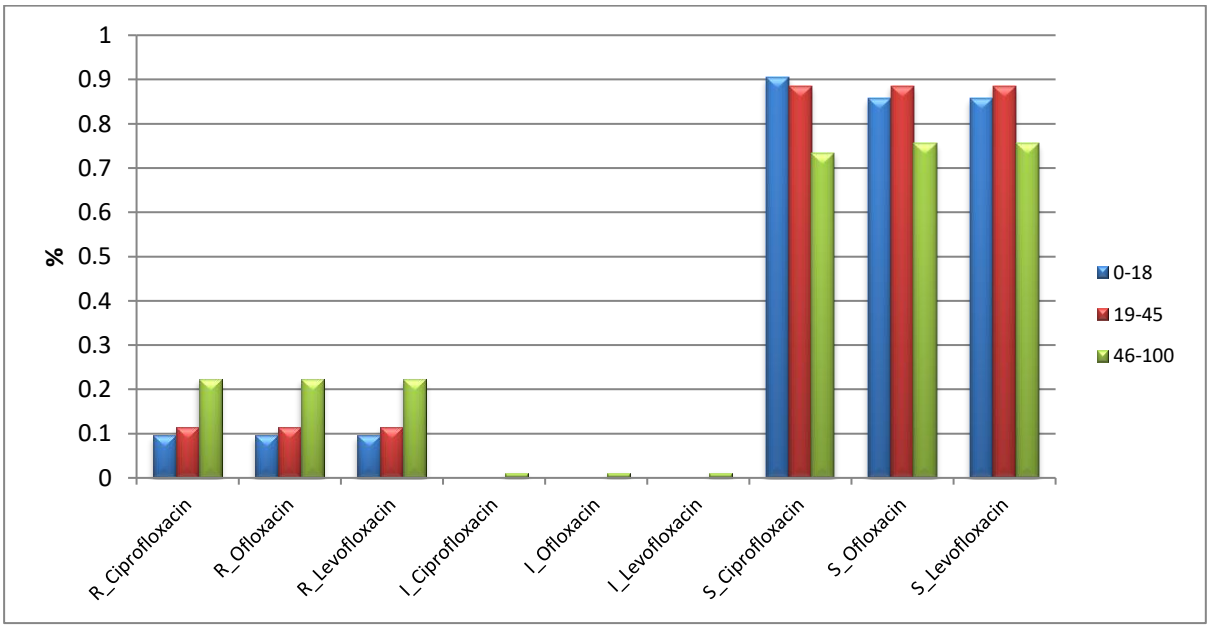


Figure 9. The sensitivity to quinolones within the tested *Enterobacteriaceae* distributed on age groups (0-100 years)

The graph pointed out a higher resistance in people over 46 years old (about 22%). In teens and adults under 46, a higher sensitivity and a lower resistance occurred. It pointed out an increase of the bacterial resistance to antibiotics along with aging, that may point out the frequent use of antibiotics as a cure without being absolutely necessary. Regarding the sensitivity/resistance of the pathogens to quinolones, it was noticed a higher susceptibility to quinolones in females (79%) compared to males (71%) (Figures 10, 11).

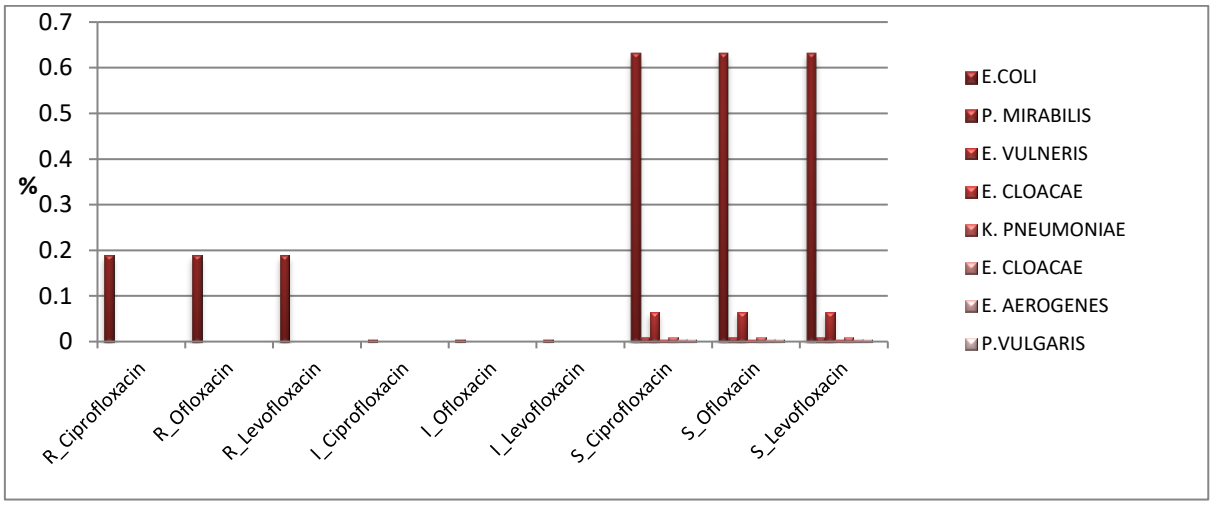


Figure 10. The sensitivity to quinolones of the main *Enterobacteriaceae* strains in women

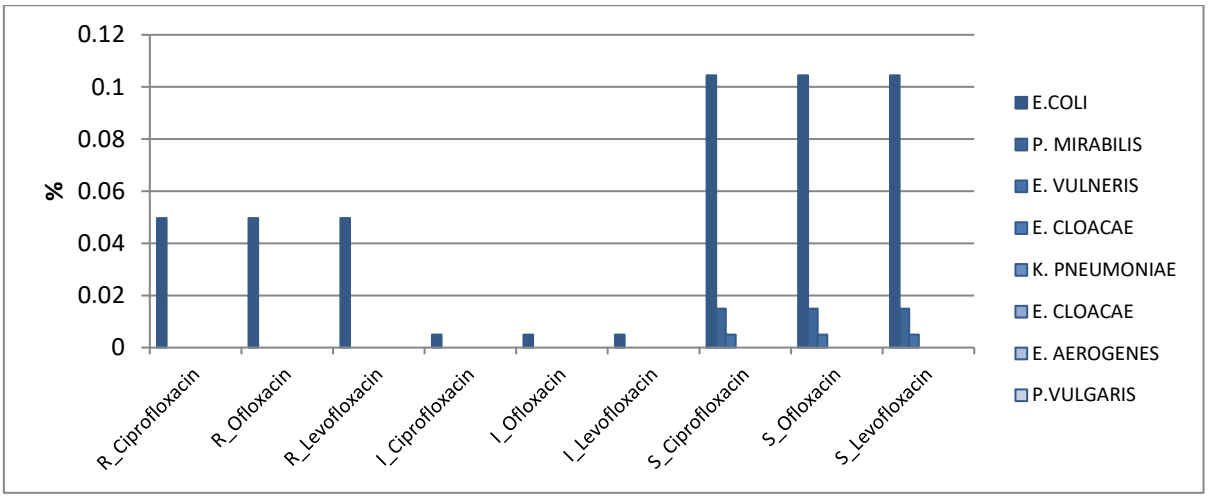


Figure 11. The sensitivity to quinolones of the main *Enterobacteriaceae* strains in men

Therefore, the pathogens that caused UTIs in women display a slightly higher sensitivity to quinolones. At the same time, one may notice that the resistance to quinolones is lower in women (20%) as compared to men (26%). This may indicate a more successful quinolone cure of the UTIs in females. The higher resistance to quinolones in males suggests the frequent use of antibiotics in various diseases or there are different types of infections, mainly recurrent ones or prostate disorders.

CONCLUSION

The survey on the distribution and number of UTIs from Moldavia during the analyzed period of time pointed out a rather good health state in the human population, based on the pathogens' frequency and the resistance patterns. For antibiotic sensitivity testing, antibiograms were performed, followed by the identification of a significant percentage of resistant strains, mainly *E. coli* strains, in accordance with the European data on the decrease of fluoroquinolones' efficiency in the treatment of UTIs. This situation is caused by an excessive use of this group of antibiotics that evinces the relevance of the lab diagnosis prior to the antibiotics' treatment.
In this respect, the priorities are an accurate diagnosis and a reasonable antibiotic therapy to prevent the occurrence of some multiresistant strains.

The intake of antibiotics in the cure of the UTIs (without prior urocultures and antibiograms) increases the risk of multiresistant bacterial strains.
The large range of fluoroquinolones' sensitivity acknowledges the increasing trend of bacterial resistance. These uropathogenic bacteria display a wide phenotypic and genotypic variability and frequently develop immunological paths of resistance to many classes of antibiotics.

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