

## ANTIBIOTIC SENSITIVITY OF THE *ESCHERICHIA COLI* STRAINS ISOLATED FROM INFECTED SKIN WOUNDS

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### Abstract

The exaggerated use of antibiotics has led to the selection of new strains of bacteria that resist to antibiotics, a situation which is found in the case of *Escherichia coli* strains. In this study we have evaluated the sensitivity to antibiotics of *E. coli* strains isolated from several types of infected wounds. This is a retrospective study on the sensitivity to antibiotics of 113 strains of *E. coli* isolated from infected wounds in patients hospitalized into the Emergency Hospital in Oradea between 2006-2008. Also, the objective of this study was to assess the accuracy of two tests used to determine the sensitivity to 4 selected antimicrobial drugs: the disk diffusion method and the agar dilution method. Strains of *E. coli* were identified based on their morpho-tinctorial, culture and especially biochemical properties, using API 20E Biomerieux tests. The antibiotic sensitivity test was performed using the disc diffusion method and the agar dilution method, prepared according to the standards of the Clinical and Laboratory Standard Institute (CLSI, 2009). The results revealed a 75% sensitivity to amikacin, 52.3% to imipenem and between 35-50% to IV-th generation cephalosporins. The lowest level of sensitivity was recorded for ampicillin (6.8%). Our attention was drawn by a lower level of sensitivity to gentamicin, that of 38.6% as compared to 70% which is found in international statistics. Sensitivity to penicillins and cephalosporins was also low, the number of resistant strains being over 50% compared to the 19.5% rate reported in the Sentry Programme in 2004. The results of disc diffusion test for *E. coli* and for the tested antibiotics were perfectly reproducible and comparable with the agar dilution method. In conclusion, the isolated *E. coli* strains are highly sensitive to the third generation aminoglycosides, fourth generation cephalosporins and carbapenem. In this study, we found the disc diffusion to be a reliable, easy and inexpensive method for testing the susceptibility of *E. coli* to: ampicillin, amikacin, ciprofloxacin and ceftazidime.

### Rezumat

Utilizarea exagerată a antibioticelor a condus la selecția de tulpini bacteriene polirezistente la antibiotice, situație ce se regăsește și în cazul tulpinilor de *Escherichia coli*. În studiul de față am urmărit sensibilitatea la antibiotice a tulpinilor de *E. coli* izolate din mai multe tipuri de plăgi infectate. Este un studiu retrospectiv asupra sensibilității la antibiotice a 113 tulpini de *E. coli* izolate din plăgi cutanate infectate de la pacienții internați în Spitalul Clinic de Urgență Oradea în perioada 2006-2008. De asemenea, obiectivul studiului a fost acela de a evalua precizia a două metode pentru determinarea sensibilității la 4 antibiotice selectate: metoda difuzimetrică și metoda

diluțiilor în agar. Tulpinile au fost identificate pe baza proprietăților morfotinctoriale, de cultură și în special biochimice, utilizând teste API 20E Biomerieux. Antibiograma s-a efectuat prin metoda difuzimetrică și metoda diluțiilor în agar conform standardului Clinical and Laboratory Standards Institute 2009 (CLSI-2009). Rezultatele obținute au evidențiat o sensibilitate de 75% la amikacină, 52,3% la imipenem și între 35-50% la cefalosporinele de generația a IV-a. Cel mai scăzut nivel de sensibilitate s-a înregistrat la ampicilină, 6,8%. A atras atenția nivelul mai scăzut de sensibilitate la gentamicină de 38,6% față de 70% după cum se regăsește în statisticile internaționale. De asemenea, sensibilitatea la peniciline și cefalosporine este redusă, numărul tulpinilor rezistente este de peste 50% față de 19,5%, procent raportat de Programul Sentry în 2004. Rezultatele metodei difuzimetrice pentru *E. coli* și antibioticele testate au fost perfect reproductibile și comparabile cu metoda diluțiilor în agar. Un procent ridicat din tulpinile de *E. coli* izolate sunt foarte sensibile la: aminoglicozide de generația a III-a, cefalosporine de generația a IV-a și carbapeneme. În acest studiu am constatat, de asemenea, că metoda difuzimetrică este o metodă fiabilă, ușoară și ieftină pentru testarea sensibilității tulpinilor de *E. coli* la: ampicilină, amikacină, ciprofloxacina și ceftazidim.

**Keywords:** Infected wounds, bacterial sensitivity, *Escherichia coli*

### Introduction

*Escherichia coli* is a gram-negative bacillus that belongs to the *Escherichia genus*, which is made up of species present in the human and other animal intestine. When eliminated in the environment together with feces it contaminates water, soil and food [1, 5].

The pathogenicity of *E. coli* is explained by its virulence and/or toxicity. The factors that determine its pathogenicity are varied and, depending on their nature, *E. coli* strains may cause various infections, including infections of the skin wounds [4, 6, 20].

Infected wounds are commonly encountered in medical practice raising issues of diagnosis and treatment because of the selection of bacterial strains resistant to antibiotics [11, 12, 18]. This situation occurs for the strains of *E. coli* [13].

Therefore our goal was to study the sensitivity to certain antibiotics of such strains isolated from several types of infected skin wounds and to establish a correlation between susceptibility and the type of wound.

Also, the objective of this study was to assess the accuracy of two tests used to determine the sensitivity to 4 selected antimicrobial drugs: the disc diffusion method and the agar dilution method.

## Materials and methods

In our study we have included 113 strains of *E. coli* isolated from infected skin wounds in patients hospitalized into the Emergency Hospital in Oradea, between 2006-2008.

*E. coli* was isolated from the bacteriological examination of purulent secretion taken from the wounds. We obtained gram-stained slides which we examined microscopically.

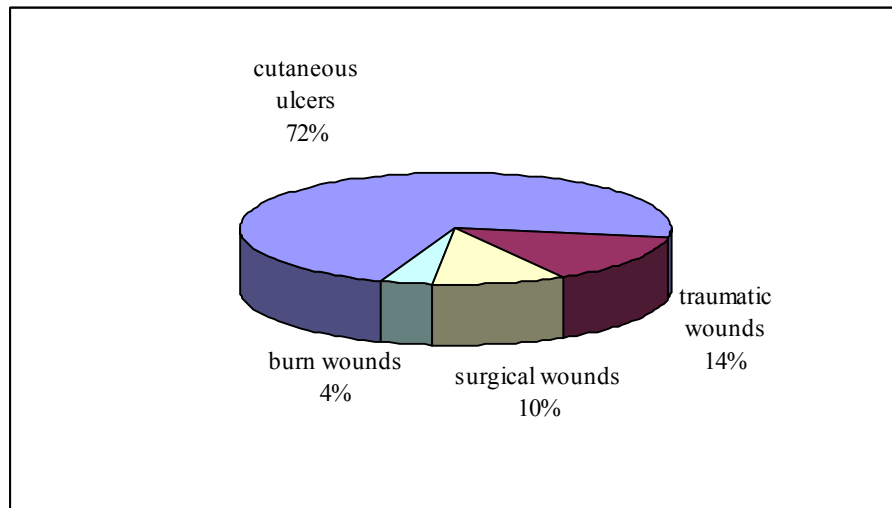
Purulent secretions were cultivated on Nutrient Broth (37°C, 24 h), and then transferred on selective media (blood-agar, Mac Conkey agar, Levin, CLED-agar) and subsequently on average multitest media (MIU, TSI, Simmons). For the final identification we used API 20E Biomerieux tests [9, 19].

For the disc diffusion testing: ampicillin, gentamicin, amikacin, augmentin, cefuroxime, cefoperazone, ciprofloxacin, imipenem, piperacillin, trimethoprim-sulfamethoxazol and ceftazidime disc were used. For the agar dilution method, the following antibiotics were tested: ampicillin, amikacin, ciprofloxacin and ceftazidime. Antibiotic sensitivity testing by both methods was applied for only 30 *E. coli* strains. The concentrations of the antibiotics tested were 0.5 – 64 µg/mL. Inocula were prepared in nutritive broth at a density adjusted to a 0.5 Mc Farland turbidity standard for disc diffusion and diluted 1:10 for the dilution method. A final inoculum was placed on Müller-Hinton agar plates. The inoculated plates were incubated at 37°C under aerophilic atmosphere for 24 h. For the agar dilution, the end point was taken as the complete inhibition of microscopic growth, for the disc diffusion, the zone diameters were measured with slipping calipers. The following control strain was used: *Escherichia coli* ATCC 25922. Susceptibility criteria for the agar dilution method and the drug concentrations in the disc were those of the CLSI [2, 3].

## Results and discussion

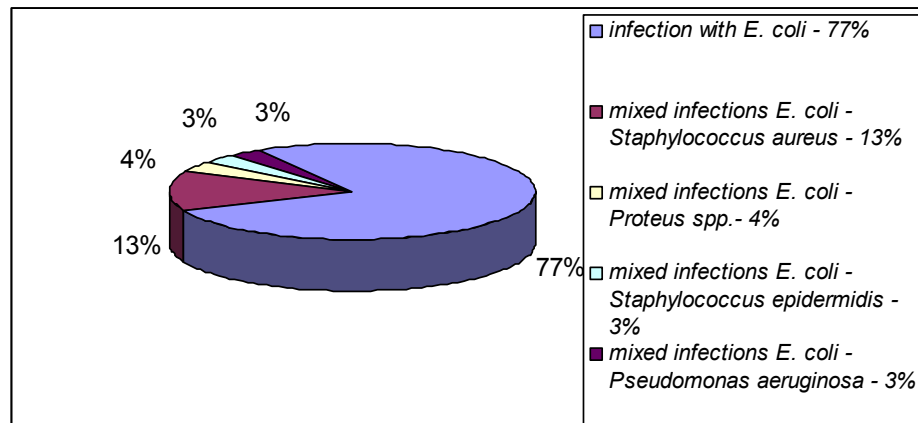
In our study group there were predominantly women, the ratio women/men is 1.3/1, resulting in a significantly higher prevalence of wound infections among women. The age of the patients was over 50.

Most strains of *E. coli* were isolated from purulent skin ulcers secretions (figure 1).



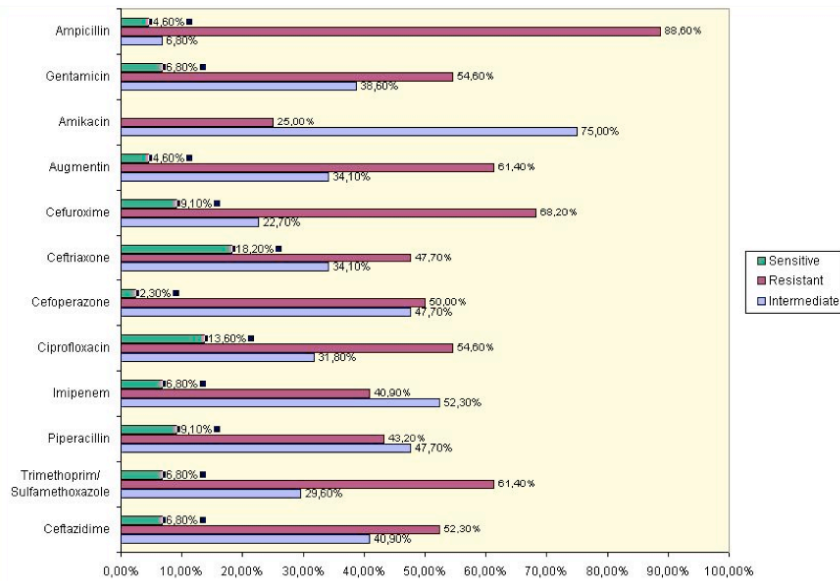
**Figure 1.**  
Sources of infections with *Escherichia coli*

Out of the 113 isolated strains of *E. coli*, 87 were identified as the single etiological agent of infected wounds and 26 strains were involved in mixed infections. The most frequent association was between *E. coli* and *Staphylococcus aureus* - 15 cases respectively 13% (figure 2).



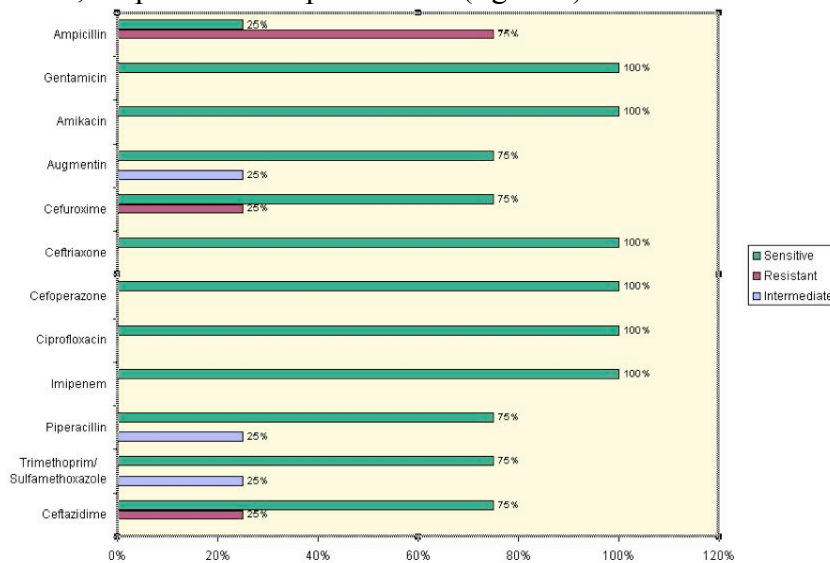
**Figure 2.**  
Types of infections with *Escherichia coli*

As for the sensitivity to antibiotics, most of the strains (75%) were sensitive to amikacin and only 6.8% were sensitive to ampicillin. Over 50% of the strains were sensitive to imipenem (figure 3).



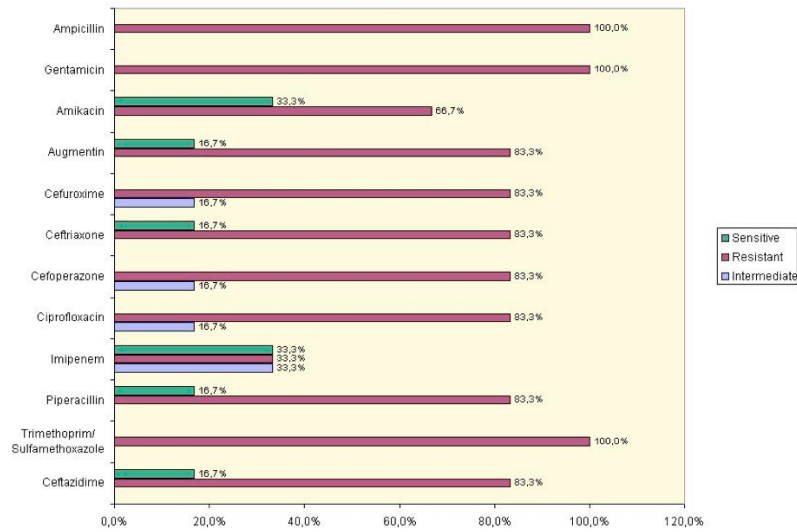
**Figure 3.**  
Sensitivity to antibiotics of *Escherichia coli* strains

We studied the sensitivity of *E. coli* strains depending on the type of wound from which they were isolated. All *E. coli* strains isolated from surgical wounds were sensitive to amikacin, gentamicin, cefoperazone, ceftriaxone, imipenem and ciprofloxacin (figure 4).



**Figure 4.**  
Response to sensitivity test of antibiotics in surgical wounds - *Escherichia coli*

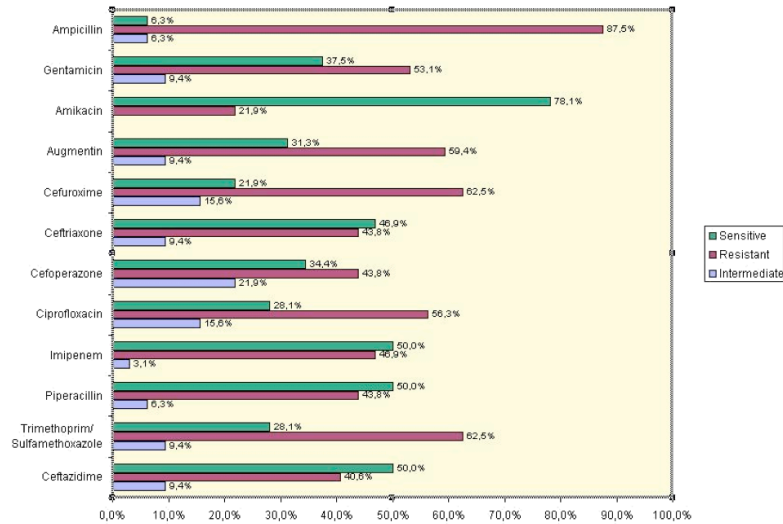
In traumatic wounds infected with *E. coli*, most strains were resistant to all antibiotics to which the testing was conducted (figure 5).



**Figure 5.**

Response to sensitivity test of antibiotics in traumatic wounds - *Escherichia coli*

The best responses in patients with chronic ulcers infected with *E. coli* were to amikacin (78.1%), followed by imipenem, piperacillin and ceftazidime (50.0%) (figure 6).



**Figure 6.**

Response to sensitivity test of antibiotics in chronic ulcers - *Escherichia coli*

*E. coli* strains involved in mixed infections had an increased resistance to antibiotics. Ceftazidime and amikacin were the only effective antibiotics in these situations.

*E. coli* strains isolated from burns also had a high degree of resistance to antibiotics.

The results of susceptibility testing using the disc diffusion and the agar dilution methods for each antibiotic are reported in table I.

**Table I.**  
Results of the susceptibility testing of 30 strains of *Escherichia coli* by disc diffusion and agar dilution method for four antibiotics

Method	Ampicillin	Amikacin	Ciprofloxacin	Ceftazidime
<b>Agar dilution method</b>	4 S ( $\leq 8$ $\mu\text{g/mL}$ ) 1 I (8-32 $\mu\text{g/mL}$ ) 25 R ( $\geq 32$ $\mu\text{g/mL}$ )	23 S ( $\leq 16$ $\mu\text{g/mL}$ ) 7 R ( $\geq 32$ $\mu\text{g/mL}$ )	12 S ( $\leq 1$ $\mu\text{g/mL}$ ) 3 I (1-4 $\mu\text{g/mL}$ ) 15 R ( $\geq 4$ $\mu\text{g/mL}$ )	12 S ( $\leq 8$ $\mu\text{g/mL}$ ) 3 I (8-32 $\mu\text{g/mL}$ ) 15 R ( $\geq 32$ $\mu\text{g/mL}$ )
<b>Disc diffusion method</b>	2 S ( $\geq 17$ mm) 4 I (>14-<16 mm) 24 R ( $\leq 13$ mm)	23 S ( $\geq 17$ mm) 7 R ( $\leq 14$ mm)	11 S ( $\geq 21$ mm) 5 I (>16-<20 mm) 14 R ( $\leq 15$ mm)	12 S ( $\geq 18$ mm) 4 I (>15-<17 mm) 14 R ( $\leq 14$ mm)
Note: The values found above represent the total number of strains. Where: S – sensitive, I – intermediate, R – resistant.				

By the disc diffusion method the results showed the highest sensitivity to amikacin and ceftazidime and the lowest sensitivity to ampicillin, when compared to agar dilution method.

When analysing the sensitivity of *E. coli* strains to antibiotics in all the collected samples, one can notice that the highest sensitivity is to amikacin, 75%. This is best seen in strains isolated from surgical wounds (100%) and cutaneous ulcers (78.1%), the latter being also the most frequent type of wounds.

It was found that there are considerable differences in terms of sensitivity to aminoglycosides. Sensitivity to amikacin is 75% compared to gentamicin which is effective against only 38.6% of the strains of *E. coli*. Other studies on this topic report a sensitivity of 88% to amikacin and 70% to gentamicin [14].

The sensitivity of *E. coli* strains is relatively low to penicillins and the sensitivity to ampicillin is the lowest (6.8%). The sensitivity of *E. coli* strains to cephalosporins is around 40%, especially to cefoperazone and ceftazidime. Decreased sensitivity to penicillins and cephalosporins is due

to the selection of beta-lactamase producing strains.  $\beta$ -Lactamases continue to be the leading cause of resistance to  $\beta$ -lactam antibiotics among gram-negative bacteria. Typically, they derive from genes plasmid-encoded  $\beta$ -lactamases for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these  $\beta$ -lactamases. TEM-1 is the most common plasmid-mediated  $\beta$ -lactamase of ampicillin resistant *E. coli* [15, 17].

The Sentry study has reported an increase from one year to another of the number of beta-lactamase producing strains, their percentage being 19.5% in 2004 [7].

The sensitivity of *E. coli* strains to carbapenems (imipenem) and quinolones (ciprofloxacin) is comparable with the data from literature, about 50% [8, 10]. Various studies have investigated the mechanism responsible for carbapenem and quinolones resistance in clinical isolates of *E. coli* and shown the resistance to be conferred by the high-level expression of a plasmid-mediated class C  $\beta$ -lactamase, in combination with the loss of an outer membrane protein [16, 21].

### Conclusions

Strains of *E. coli* are sensitive to third generation aminoglycosides, to fourth generation cephalosporins and to carbapenems. We could not establish a correlation between the sensitivity to antibiotics and the type of wound from which we isolated the strain.

In this study, we found the disc diffusion to be a reliable, easy and inexpensive method for testing the susceptibility of *E. coli* to: ampicillin, amikacin, ciprofloxacin and ceftazidime.

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*Manuscript received: September 28<sup>th</sup> 2009*