**ORIGINAL ARTICLE**

# COMPARATIVE STUDY OF ANTIMICROBIALS USE AND THE ANTIBIOTIC RESISTANCE OF GRAM NEGATIVE STRAINS

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**Abstract**

The aim of this study was to perform a comparative evaluation of antibiotics use in the Surgery Department of Timişoara Regional Hospital, Romania, in the first three consecutive months of the year 2015, comparatively with the same interval of the year 2016. For every patient was set up a personal record containing information about: age, diagnosis, pathological product collected, the identified germ, antibiogram, pharmaceutical form of administered antibiotic, dose, number of administrations/day, duration of therapy. Antibiotics specific intake was calculated according to the Defined Daily Dose (DDD)/1000 patients/day. It was revealed, for the monitored antibiotics classes, an increase in the use of fluoroquinolones, accompanied by a decrease of the prescriptions for the other classes. This study also revealed a decrease in the use of cephalosporins, aminoglycosides and carbapenems in the first trimester of 2016, compared with the similar interval of the year 2015 and a significant increase of the resistance of *E. coli* and *Klebsiella* spp. for gentamicin in 2016 compared with 2015.

**Rezumat**

Scopul acestui studiu a fost de a evalua comparativ consumul de antibiotice administrate pacienților într-o secție de chirurgie a Spitalului Județean Timișoara, România în primele trei luni ale anului 2015, comparativ cu același interval din 2016. Pentru fiecare pacient s-a întocmit o fișă cu următoarele informații: vârstă, diagnostic, produsul patologic colectat, agentul patogen, antibiogramă, antibioticul administrat, doza, numărul de doze/zi, durata tratamentului. Consumul de antibiotice s-a calculat folosind doza zilnică stabilită (DDD)/1000 pacienți/zi. În cazul claselor de antibiotice monitorizate este relevantă creșterea consumului de fluorochinolone, în condițiile scăderii prescrierii altor clase. Studiul demonstrează de asemenea scăderea consumului de cefalosporine, aminoglicozide și carbapeneme în primul trimestru din 2016, comparativ cu intervalul similar din 2015 și creșteri semnificative ale rezistenței la gentamicină a tulpinilor de *E. coli* și *Klebsiella spp.* în 2016 comparativ cu 2015.

**Keywords**: antibiotics use, resistance, DDD, fluoroquinolones, cephalosporins, aminoglycosides, carbapenems

## Introduction

One of the major problems for contemporary clinical practice is the huge increase in the prevalence of antibiotics resistant pathogenic bacteria [1, 3, 4, 13]. The pharmaceutical industry has reached a *plateau* concerning the production and marketing of new antimicrobial agents directed against new bacterial target - receptors. Most of the efforts in the field of antibiotherapy are directed to adjust the antibiotic dose, tolerability and also to increase the efficiency of antibiotics classes existing today on the market. Due to this situation, it is crucial to optimize the use of existing antibiotics and formulate strategies to minimize the resistance to antibiotics [8, 9]. Hospital infections caused by multiresistant bacteria are an important source of morbidity and mortality and an economic burden for health care facilities, with major impact on the whole society [5, 6, 7].

The study aimed to assess the relative consumption of antibiotics in the surgical departments in the first trimester of 2015, compared to the same period of 2016 and the evolution of antibiotic-resistant Gram negative strains isolated.

## Materials and Methods

The trial involved two groups of patients: first group included a number of 352 patients hospitalized in the Surgery Department of Timişoara Regional Hospital,

Romania, during the first trimester of 2015 and the second group included 314 patients hospitalized in the same unit, during the first three months of 2016. For each patient it was drawn up a personal record that included: age, diagnosis, the pathologic product, the germs identified, the antibiogram, the administered antibiotic, (pharmaceutical form, dosage, number of doses/day, duration of therapy). The antibiotic use was calculated using the DDD/1000 patients/day. Defined Daily Dose (DDD) is a measuring unit internationally recognized representing the mean daily dose of antibiotic administered to one adult weighing 70 kg [15].

The classes of antibiotics analysed were: third generation cephalosporins, fluoroquinolones, aminoglycosides and carbapenems, prescribed in the therapy and prophylaxis of hospital infections produced by Gram-negative bacteria. The frequency of the positive pathological samples was: sputum, peritoneal fluid, fluids drained from joints and pleura, tracheo-bronchic aspirates, secretions of lesions, central catheter, urine.

For the antibiotics we are referring to, the DDD used in our calculations is presented in Table I.

**Table I**

Defined Daily Dose for the antibiotics used in the trial

| Antibiotic | DDD | Measuring unit | Route of administration |
|---|---|---|---|
| ceftriaxone | 2 | g | parenteral |
| cefuroxime | 3 | g | parenteral |
| cefotaxime | 4 | g | parenteral |
| ceftazidime | 4 | g | parenteral |
| ciprofloxacin | 0.5 | g | parenteral |
| norfloxacin | 0.8 | g | oral |
| gentamicin | 0.24 | g | parenteral |
| amikacin | 1 | g | parenteral |
| imipenem + cilostatin | 2 | g | parenteral |

The statistic interpretations were performed using the software EPI-INFO version 7, $p \leq 0.05$ was considered statistically significant.

## Results and Discussion

After the centralization of all records for the patients hospitalized between 01.01.2015 – 31.03.2015 (sample A) and 01.01.2016 – 31.03.2016 (sample B), it was observed a decrease of the percentage for the patients undergoing an antibiotherapy with the antibiotics mentioned above in 2016 compared with 2015.

Exceptions for this rule were represented by the superior, statistically significant percentages for the subjects treated with cefuroxime and ciprofloxacin. Statistically significant decreases were recorded for the patients treated with ceftriaxone, ceftazidime and gentamicin, as seen in Table II.

**Table II**

Proportion of patients undergoing an antibiotherapy with the antibiotics studied in the study

| Antibiotic | Patients Sample A | | Patients Sample B | | p - value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| ceftriaxone | 163 | 46.30 | 66 | 21.02 | < 0.001 |
| cefuroxime | 11 | 3.12 | 24 | 7.64 | 0.009 |
| cefotaxime | 16 | 4.54 | 8 | 2.54 | Ns |
| ceftazidime | 27 | 7.67 | 6 | 1.91 | < 0.001 |
| ciprofloxacin | 9 | 2.55 | 21 | 6.68 | 0.01 |
| norfloxacin | 5 | 1.42 | 1 | 0.32 | Ns |
| gentamicin | 94 | 26.70 | 34 | 10.82 | < 0.001 |
| amikacin | 11 | 3.12 | 6 | 1.91 | Ns |
| imipenem + cilostatin | 37 | 10.51 | 23 | 7.32 | Ns |
| Total no. of patients | 352 | 100 | 314 | 100 | |

Ns = not significant

The total amount of antibiotics used in the 2 intervals of time studied is presented in Table III.

**Table III**

The intake of antibiotics in the two intervals mentioned above

| Antibiotic Class | Antibiotic | Quantities (numeric units ) | |
|---|---|---|---|
| | | Sample A | Sample B |
| cephalosporins | ceftriaxone | 1033 v (1 g) | 331 v (1 g) |
| | cefuroxime | 31 v (750 mg) | 68 v (750 mg) |
| | cefotaxime | 43 v (1 g) | 22 v (1 g) |
| | ceftazidime | 52 v (1 g) | 12 v (1 g) |

| Antibiotic Class | Antibiotic | Quantities (numeric units ) | |
|---|---|---|---|
| | | Sample A | Sample B |
| fluoroquinolones | ciprofloxacin | 30 v (1 mL) | 83 v (1 mL) |
| | norfloxacin | 16 cps (400 mg) | 4 cps (400 mg) |
| aminoglycosides | gentamicin | 378 v (40 mg) | 130 v (40 mg) |
| | amikacin | 58 v (2 mL) | 22 v (2 mL) |
| carbapenems | imipenem + cilostatin | 132 v (120 mL) | 72 v (120 mL) |

v = vials, cps = capsules

The calculus of DDD/1000 patients/day for any antibiotic mentioned in the study revealed a more frequent use of cefuroxime and ciprofloxacin, while prescribing the other antibacterial agents decreased, as it can be seen in Table IV.

**Table IV**

The use of antibiotics in the first trimester of 2015 *versus* the first trimester of 2016

| Antibiotic | DDD/1000 patients/day Trim I 2015 | DDD/1000 patients/day Trim I 2016 | Trend |
|---|---|---|---|
| ceftriaxone | 16.30 | 5.85 | ↓ with 64.12% |
| cefuroxime | 0.24 | 0.60 | ↑ with 150% |
| cefotaxime | 0.339 | 0.173 | ↓ with 48.97% |
| ceftazidime | 0.41 | 0.106 | ↓ with 74.15% |
| ciprofloxacin | 0.189 | 0.58 | ↑ with 206.87% |
| norfloxacin | 0.25 | 0.07 | ↓ with 72% |
| gentamicin | 1.99 | 0.768 | ↓ with 61.41% |
| amikacin | 0.91 | 0.389 | ↓ with 57.26% |
| imipenem + cilostatin | 1.04 | 0.636 | ↓ with 38.85% |

The comparative analysis of the global use, on monitored antibiotics classes, reveals an increase in the use of fluoroquinolones, accompanied by a decrease of the prescriptions for the other classes, as it can be seen in Table V.

**Table V**

General use for the monitored antibiotics classes in the first trimester of 2015 *versus* the first semester of 2016

| Antibiotic Class | DDD/1000 patients/day Trim I 2015 | DDD/1000 patients/day Trim I 2016 | Trend |
|---|---|---|---|
| cephalosporins | 17.289 | 6.729 | ↓ with 61.08% |
| fluoroquinolones | 0.439 | 0.65 | ↑ with 48.06% |
| aminoglycosides | 2.9 | 1.157 | ↓ with 61.11% |
| carbapenems | 1.04 | 0.636 | ↓ with 38.85% |

Also the number of days of antibiotherapy specific to any class of antibiotics considered decreased in 2016 compared to 2015, except fluoroquinolones, especially ciprofloxacin (Table VI).

**Table VI**

The duration of antibiotherapy with the monitored classes of antibiotics

| Antibiotic Class | Nr. of days of antibiotherapy Trim I 2015 | Nr. of days of antibiotherapy Trim I 2016 | Trend |
|---|---|---|---|
| cephalosporins | 502 | 192 | ↓ with 61.76% |
| fluoroquinolones | 18 | 43 | ↑ with 138.88% |
| aminoglycosides | 211 | 76 | ↓ with 63.99% |
| carbapenems | 61 | 36 | ↓ with 40.99% |

Regarding the Gram negative species isolated in the two periods taken into study, it was observed a statistically significant decrease for the strains of *E. coli*, while the frequencies of other species remained constant, as it can be seen in Table VII.

**Table VII**

Strains of Gram negative bacteria isolated in the two periods taken into study

| Species | Trim I 2015 | | Trim I 2016 | | p |
|---|---|---|---|---|---|
| | N | %* | N | %* | |
| *E. coli* | 15 | 28.30 | 4 | 9.30 | **0.02** |
| *Klebsiella* spp. | 25 | 47.17 | 28 | 65.11 | Ns |
| *Proteus* spp. | 7 | 13.20 | 7 | 16.28 | Ns |
| *Enterobacter* spp. | 1 | 1.89 | 0 | 0 | Ns |
| **TOTAL *Enterobacteriaceae*** | 48 | 90.56 | 39 | 90.69 | Ns |
| *Acinetobacter* spp. | 1 | 1.89 | 1 | 2.32 | Ns |
| *Pseudomonas* spp. | 4 | 7.54 | 3 | 6.97 | Ns |
| **TOTAL Non-fermentative** | 5 | 9.43 | 4 | 9.30 | Ns |
| **TOTAL** | 53 | 100 | 43 | 100 | |

* % of the total of G negative bacteria isolated, Ns = not significant

Concerning the resistance of Gram negative strains for the studied periods, statistics revealed significant increases of the resistance of *E. coli* and *Klebsiella spp.* for gentamicin in 2015 compared with 2016. In the case of ciprofloxacin, even if the use increased in 2016 compared with 2015, it was not documented any increase of resistance for Gram negative germs (Table VIII)

**Table VIII**

The evolution of antibiotic resistance for the isolates strains

| **Antibiotic** | ***Proteus* spp.** | | | ***Pseudomonas* spp.** | | | ***E. coli*** | | | ***Klebsiella* spp.** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **%R* 2015** | **%R 2016** | **p** | **%R 2015** | **%R 2016** | **p** | **%R 2015** | **%R 2016** | **p** | **%R 2015** | **%R 2016** | **p** |
| cefuroxime | 57.14 | 57.14 | Ns | | | | | | | | | |
| ceftriaxone | | | | | | | | | | 4 | 3.75 | Ns |
| ciprofloxacin | 57.14 | 57.14 | Ns | 50 | 66.66 | Ns | 46.66 | 100 | Ns | 60 | 60.71 | Ns |
| norfloxacin | 57.14 | 57.14 | Ns | | | | | | | | | |
| gentamicin | | | | | | | 6.66 | 75 | **0.015** | 16 | 85.71 | **< 0.001** |
| imipenem + cilostatin | 14.28 | 14.28 | Ns | 50 | 66.66 | Ns | | | | 24 | 3.57 | **0.04** |

R* = Resistance, Ns = not significant

Several European studies aimed to assess the complex impact of antibiotherapy controlling program, reflected by the diminished hospitalization duration, decreasing the mortality rate, decreasing the costs and the influence over bacterial resistance and nosocomial infections [10, 11, 12]. A detailed calculus of DDD/1000 patients/day for any department may have significant results.

European Study on Antibiotic Consumption (ESAC) collects data regarding the antibiotics use in 24 European countries, through nationally coordinated networks [5].

The most recent data concerning *per os* administered antibiotics in Romania reveals an extensive use of wide spectrum penicillin (amoxicillin, amoxicillin + clavulanic acid, ampicillin etc.), of penicillin with clavulanic moderate or narrow spectrum and relatively equal proportions of macrolides, tetracyclines and sulfametoxazol + trimethoprim [12]. Injectable antibiotic ceftriaxone was the most used, as underlined in the present study. European Antimicrobial Resistance Surveillance System (EARSS) contains data collected since 1999 from 27 countries, referring to resistance on *E. coli*, *S. pneumoniae, Staphylococcus aureus and Enterococcus faecalis/ faecium.* EARSS is financed by The European Commission, coordinated by National Institute of Public Health and Environment from Holland (RIVM) and continues to collect data in present [5]. Romania participates to this project since 2002 by the laboratories belonging to university centres and regional hospitals, serving together about half of the population.

Similar data were gathered during some other studies performed in other geographical areas, regarding the surveillance for antibiotic resistance [11, 12].

A mutual conclusion of the studies mentioned above is that the proportion of antibiotics resistant bacterial strains presents large variations in different countries, most probably due to the differences between the consumption of antibiotics and hospital infection control [15].

As consequence, the planning of bacterial resistance combating must be specifically adapted at the country level, region and hospital, based on collected data [2, 16].

The present study highlights the statistically significant reduction of *E. coli* strains, which can mean appropriate measures to control nosocomial infections, and also an increase in resistance of *Klebsiella* spp. strains to gentamicin, in 2016 compared to 2015.

## Conclusions

The use of cephalosporins, aminoglycosides and carbapenemes decreased in the first trimester of 2016 compared with the similar period of 2015, by reducing the number of patients undergoing such treatment and consecutively by reducing the number of days of hospitalization. The consumption of quinolones increased, based on the increase in the intake of Ciprofloxacin, due to its effectiveness against the Gram negative strains.

The distribution of *Enterobacteriaceae* and non-fermentative species is similar for the two intervals analysed, excepting a statistically significant decease for *E. coli* strains, which can signify adequate measures to control hospital infections.

It was recorded an increase of *Klebsiella* spp. strains resistance to gentamicin, in 2016 compared with 2015. For *E. coli* strains, the statistically significant value of was influenced by the small number of strains isolated during 2016.

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