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ASSESSMENT OF THE SENSITIVITY TO CARBAPENEMS OF GRAM-NEGATIVE BACILLI ISOLATED FROM HOSPITALIZED PATIENTS

Mihaela Elena IDOMIR[1,2] *

Abstract: *The study was retrospective and aimed to dynamically assess the sensitivity to carbapenems (imipenem, meropenem) of Gram-negative bacilli isolated from various clinical specimens collected from patients admitted in the Clinical County Emergency Hospital of Brasov between 1.01.2016-31.12.2020. High levels of resistance were recorded in the case of Providencia spp. (> 80%), Acinetobacter species (> 70% to imipenem; > 80% to meropenem) and Pseudomonas aeruginosa (25% -50%). The study highlights the importance of monitoring carbapenem resistance in order to optimize the etiological therapy of infections with these bacteria.*

Key words: *carbapenems, carbapenem-resistant Enterobacteriaceae, multi-drug-resistance, infections*

1. Introduction

Penicillin, discovered by Alexander Fleming in 1928 and used in therapy in 1941, was the first beta-lactamic antibiotic which initially proved to be a powerful weapon especially in the case of Gram-positive germ infections. Fleming warned that bacteria could become resistant and recommended discreet use of the antibiotic. The first penicillin-resistant strains of *Staphylococcus aureus* were reported after about one year of clinical use. A plasmid gene, blaZ, capable of encoding a beta-lactamase-like enzyme called penicillinase could be identified in such strains. This was inactivating the antibiotic by cleaving the beta-lactam ring [1],[2].

In the last 3 decades, new beta-lactam antibiotics have been introduced, developed from the 6-aminopenicillanic acid, the basic compound of penicillin, or naturally discovered, this family of antimicrobials including today's penicillins and their derivatives, generation I, II, III and IV of

[1] Transilvania University of Brasov, Faculty of Medicine
[2] Clinical County Emergency Hospital Brasov
*corresponding author: mihaela.idomir@unitbv.ro

cephalosporins, carbapenems, monobactams and β-lactamase inhibitors.

The mechanism of action of beta-lactams consists in their binding to PLP (penicillin-binding proteins), which are enzymes involved in the synthesis of peptidoglycan, a polymer in the structure of the bacterial cell wall. By binding, it inhibits the enzymatic action (trans- or carboxypeptidases, trans-glycosylases) which leads to the formation of a thin layer of peptidoglycan and a deficient cell wall, followed by bacterial cytolysis [3].

In the late 1960s, bacterial synthesis of beta-lactamases became a serious threat to penicillin therapy, intensifying efforts to discover beta-lactamase inhibitors. By 1976, olivanic acids were discovered, secreted by Streptomyces clavuligerus, structural precursors of carbapenems, broad-spectrum but abandoned due to chemical instability and poor penetrability in the bacterial cell. Clavulanic acid was obtained from the same bacterial species while thienamycin from Streptomyces cattleya, the latter being the basic compound from which all carbapenems were derived [2].

Carbapenems (imipenem / cilastatin, meropenem, ertapenem, doripenem) are considered beta-lactams with bactericidal action and broad spectrum of action. Their molecular structure gives them remarkable stability to most β-lactamases, including broad-spectrum β-lactamases (ESBL). Therefore, they are considered reserve antibiotics for infections caused by multidrug-resistant pathogens (ESBL and AmpC producers Enterobacteriaceae, multidrug-resistant Acinetobacter and Pseudomonas strains), the emergence and alarming spread of resistance to them becoming a serious public health threat [4].

Compared to imipenem, meropenem is more active on Gram-negative bacilli and less active on Gram-positive cocci (especially *Enterococcus species*). Doripenem is similar to meropenem but more active on *Pseudomonas species*. Ertapenem is less active on *Pseudomonas spp., Acinetobacter spp.* and *Enterococcus spp.* [1], [2], [5].

Infections caused by Enterobacteriaceae CRE (Carbapenem Resistant Enterobacteriaceae) are associated with high morbidity and mortality rates. CDC (Centers for Disease Control and Prevention) defines CRE as being in vitro resistant Enterobacteriaceae to any carbapenem, this phenotype including both carbapemenase-producing and non-carbapemenase-producing strains. The most common types of carbapemenases are KPC (Klebsiella pneumoniae Carbapenemase), a class A carbapenemases, especially KPC-2 and KPC-3. NDM (New Delhi Metallo-β-lactamases), class B and OXA-48, class D are also often involved.

Other mechanisms by which Enterobacteriaceae acquire resistance to carbapenems are efflux pumps, porin loss and target modifications [6 - 7].

CRE are most commonly involved in urinary tract infections but also in bloodstream infections and pneumonia, with high mortality rates, especially in immunocompromised patients. In *Pseudomonas aeruginosa* and *Acinetobacter spp.*, resistance to carbapenems is due to low membrane permeability or the association of multiple resistance mechanisms (carbapemenases, deficiency or repression of the porin, overexpression of efflux pump) [7 - 10].

2. Material and Methods

The study on the acquired carbapenem resistance of Gram-negative bacilli was

retrospective, descriptive, the analyzed data being obtained from the WHO-net database used in the bacteriological department of the laboratory of the Clinical County Emergency Hospital of Brasov in the period 1.01.2016-31.12.2020.

The inclusion criteria of the bacterial strains in the study group were the possibility to identify them by manual / automatic biochemical tests and to perform a diffusimetric / automatic antibiogram. Strains from outpatients with no pathogenic significance or repeatedly isolated from the same patient and from the same pathological product were excluded.

The Kirby-Bauer diffusimetric technique was used to test the bacterial susceptibility to antibiotics, the results being interpreted and reported according to CLSI guide (Clinical Laboratory Standard Institute) from 2016-2020. Among carbapenems, imipenem and meropenem (10 µg) were tested. For the multidrug-resistant strains, in some cases, an automatic antibiogram was performed with the VITEK 2 COMPACT system.

3. Results and Discussions

The study aimed to dynamically evaluate the share of imipenem and meropenem-resistant strains for various generes/ species of Gram-negative bacilli, isolated from pathological products collected from hospitalized patients during the study period.

Genres from the Enterobacteriaceae family were analysed, as well as the non-fermentative bacilli (*Acinetobacter spp., P. aeruginosa*) isolated during the studied period (Table 1). In the case of *Proteus* and *Providencia*, only meropenem results are shown, and their intrinsic resistance to imipenem is known [7].

The results obtained for the strains that were tested for carbapenems are illustrated in Figures 1-10.

Table 1

Gram negative bacilli tested to carbapenems

Bacterial genre	No. of strains
Escherichia coli	7531
Klebsiella spp.	2324
Proteus spp.	1768
Providencia spp.	401
Enterobacter spp.	123
Serratia spp.	41
Citrobacter spp.	38
Acinetobacter spp.	1394
Pseudomonas spp.	2106

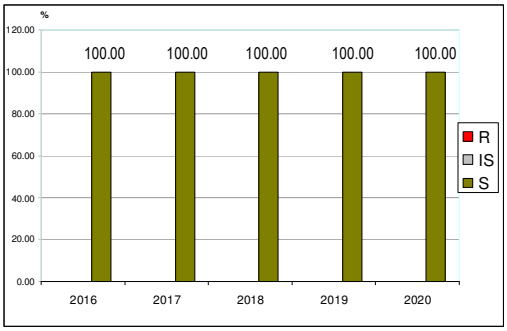

Fig. 1. *Imipenem-resistant Escherichia coli*

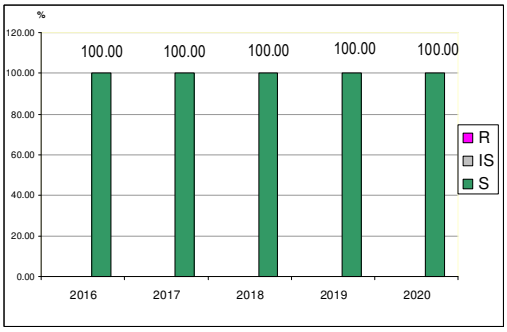

Fig. 2. *Meropenem-resistant Escherichia coli*

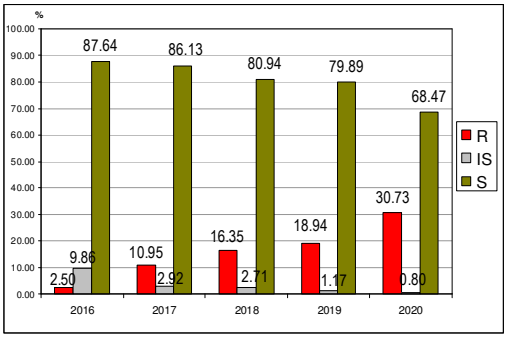

Fig. 3. *Imipenem-resistant Klebsiella spp.*

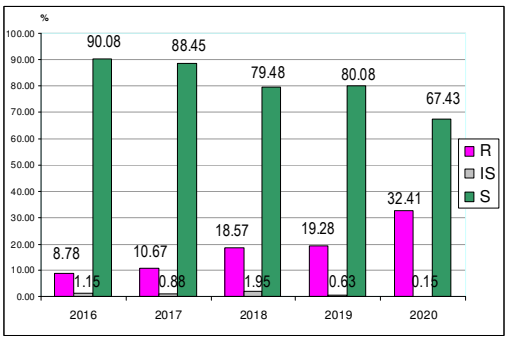

Fig. 4. *Meropenem-resistant Klebsiella spp.*

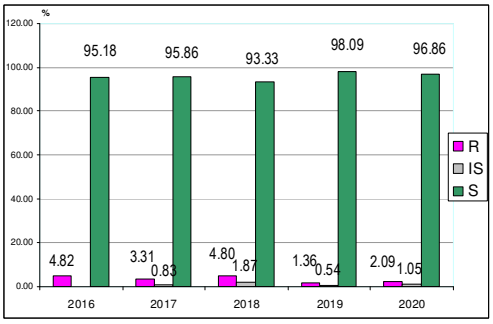

Fig. 5. *Meropenem-resistant Proteus spp.*

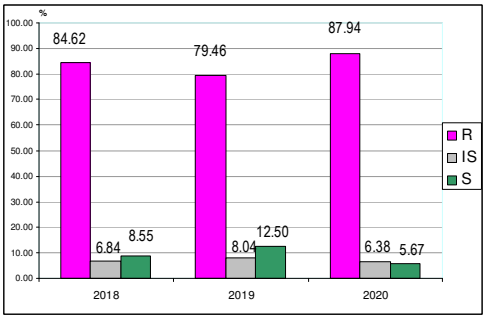

Fig. 6. *Meropenem-resistant Providencia spp.*

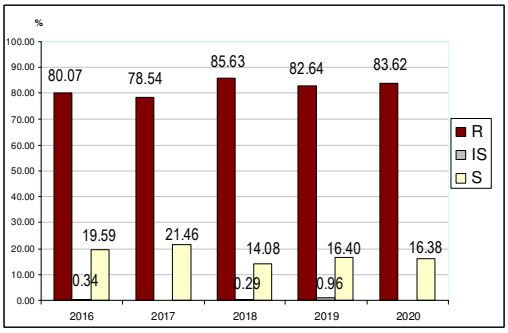

Fig. 7. *Imipenem-resistant Acinetobacter spp.*

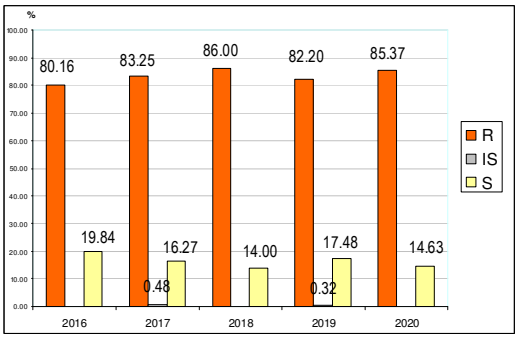

Fig. 8. *Meropenem-resistant Acinetobacter spp.*

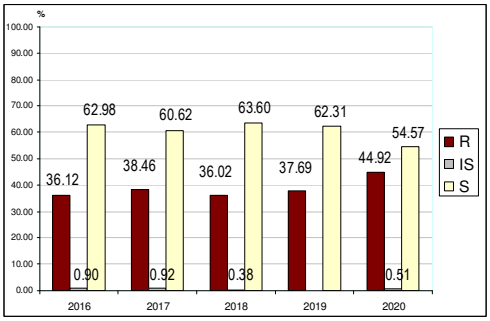

Fig. 9. *Imipenem-resistant P. aeruginosa*

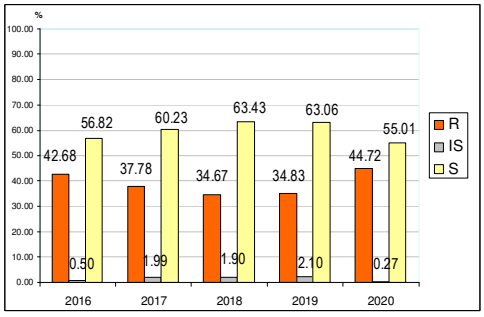

Fig. 10. *Meropenem-resistant P. aeruginosa*

In figures 1-10 there were used following qualifications, reported after performing the antibiogram:

S = sensitive to antibiotic
IS = intermediate-sensitive to antibiotic
R = resistant to antibiotic

Enterobacter spp., Serratia spp. and *Citrobacter spp.* were not analyzed because the number of tested strains was small.

The results of the study are in line with those published by ECDC (European Center for Disease Prevention and Control) reports for Romania for the period 2016-2020, which show percentages of carbapenem-resistant strains below 1% in *Escherichia coli*, between 25% and 50% in *Klebsiella pneumoniae* and over 50% in *Acinetobacter species* and *Pseudomonas aeruginosa*. The 2020 report shows that, at European level, the rate of antibiotic resistance is higher for the south-eastern regions. In the case of carbapenems, resistance is rare for of *E. coli*, in the case of *Klebsiella spp.* it is over 25% in 30% of the countries and *for Acinetobacter spp.* and *Pseudomonas aeruginosa* the resistance is common and in higher percentages than in *Klebsiella spp*. [11-15].

Other studies report variable results in *Klebsiella spp*. and low percentages in *E. coli*. [16-17]. Most carbapenem-resistant *Klebsiella spp*. strains came mainly from ICU (53,33%) and Internal Diseases (13,04%). The most common pathological products have been urine (43,33%), respiratory secretions (18,26%), wound secretions (%) and pus (10,72%).

Carbapenem-resistant strains of *Providencia spp*. came mainly from ICU (75,64%). Isolations were more common in respiratory secretions (33,01%), urine (21,15%) and pus (19,23%).

Most carbapenem-resistant strains of *Pseudomonas aeruginosa* were from ICU (45,57%), Plastic Surgery (14,58%), Internal Diseases (9,5%) and General Surgery (6,25%). Isolations were more frequent in respiratory secretions (24,08%), urine (23,56%), wound secretions (23,04%) and pus (15,23%).

Carbapenem-resistant *Acinetobacter spp.* strains came mainly from ICU (57,14%), Plastic Surgery (11,11%) and General Surgery (7,18%). The most common pathological products have been respiratory secretions (33,7%), pus (25,77%) and wound secretions (23,52%).

4. Conclusions

During the study period, no carbapenem-resistant strains were identified in the case of *E. coli, Enterobacter spp., Serratia spp., Citrobacter spp*.

The weight of carbapenem-resistant strains of *Proteus spp.* was very small, the results being able to be influenced by the difficulties of differentiation by manual biochemical tests of *Providencia spp*.

In *Klebsiella spp*., the share of resistant strains increased steadily from one study year to the next for both carbapenems.

High shares of carbapenem-resistant strains were observed in *Providencia spp*. (over 80% to meropenem), in *Acinetobacter spp*. (over 70% to imipenem and over 80% to meropenem) and *Pseudomonas spp*. (25% -50%).

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