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***ENTEROBACTERIACEAE* STRAINS WITH REDUCED SUSCEPTIBILITY  
TO CARBAPENEMS**

**SZCZEPY *ENTEROBACTERIACEAE* O OBNIŻONEJ WRAŻLIWOŚCI  
NA KARBAPENEMY**

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**S u m m a r y**

The aim of the study was to estimate the prevalence of *Enterobacteriaceae* with reduced susceptibility to carbapenems. Forty-five strains of *Enterobacteriaceae* with reduced susceptibility to carbapenems were investigated. The strains were cultured from patients treated at the Dr Antoni Jurasz University Hospital no.1 in Bydgoszcz. Identification of the strains was carried out based on the results of biochemical reactions performed in identification cards GN (bioMérieux). Susceptibility testing to carbapenems and determining Minimal Inhibitory Concentration (MIC) was

carried out using E - test (bioMérieux) (for ertapenem and imipenem), and agar dilution method for meropenem. The analysed strains were isolated from: urine – 13 strains (28.9%), blood and bronchoalveolar lavage - six strains from each (13.3% ), 5 – wound swabs (11.2%) and other clinical materials – 15 (33.3%). 53.3% of the strains were identified as *Klebsiella pneumoniae*. 93.4% of the isolates were susceptible to meropenem. Only 4.5% of the analysed strains were susceptible to ertapenem. None of the analyzed strains produced carbapenemases.

**S t r e s z c z e n i e**

Celem pracy była ocena występowania szczepów z rodziny *Enterobacteriaceae* o obniżonej wrażliwości na karbapenemy. Badaniem objęto czterdzieści pięć szczepów z rodziny *Enterobacteriaceae* o obniżonej wrażliwości na karbapenemy. Badane szczepy zostały wyizolowane od pacjentów leczonych w Szpitalu Uniwersyteckim nr 1 im. dr. Antoniego Jurasza w Bydgoszczy. Drobnoustroje zidentyfikowano na podstawie reakcji biochemicznych z wykorzystaniem kart identyfikacyjnych GN (bioMérieux). Określenie wrażliwości na karbapenemy oraz najmniejszego stężenia hamującego (MIC – Minimal Inhibitory Concentration) przeprowadzono metodą E-testu (bioMérieux)

(ertapenem i imipenem) oraz metodą rozcieńczeń w podłożu stałym (meropenem). Analizowane szczepy wyizolowane zostały odpowiednio z: moczu – 13 szczepów (28,9%), krwi i płucznin oskrzelowo-pęcherzykowych – odpowiednio po 6 (13,3% ), 5 – wymazu z ran (11,2%) oraz z innych materiałów biologicznych – 15 (33,3%). Ponad połowę badanych 53,3% szczepów zidentyfikowano jak *Klebsiella pneumoniae*. 93,4% badanych szczepów było wrażliwych na meropenem. Jedynie 4,5% szczepów było wrażliwych na ertapenem. Żaden z analizowanych szczepów nie produkował karbapenemaz.

**Key words:** *Enterobacteriaceae*, carbapenem-resistant *Enterobacteriaceae*, reduced susceptibility to carbapenems **Słowa kluczowe:** *Enterobacteriaceae*, *Enterobacteriaceae* oporne na karbapenemy, obniżona wrażliwość na karbapenemy

## INTRODUCTION

*Enterobacteriaceae* rods are a part of human bacterial flora. More and more often they are the etiological agents of both hospital and outpatient infections. In recent years, many authors have observed a significant increase of pathogens resistance to antimicrobiological agents [1, 2, 3]. In the case of multidrug resistant strains, carbapenems are more and more frequently the only therapeutic option [4]. In relation to other beta - lactams these antibiotics are characterized by the widest substrate spectra; they are active against Gram - positive and Gram - negative bacteria as well as against some anaerobes. In regard to the increasingly frequent need of carbapenems use in the treatment of multidrug strains infections, an increase in the number of isolated carbapenem - resistant *Enterobacteriaceae* (CRE) strains can be observed [5, 6, 7, 8]. Spread of CRE poses a serious threat to public health because of resulting infections. They are difficult to treat and result in high mortality. Resistance to carbapenems may be related to different mechanisms, such as production of beta - lactamases, active pumping out a drug (efflux pumps) or mutations encoding the expression of PBP. There are also strains that produce more than one mechanism of resistance.

The aim of the study was to determine the prevalence of *Enterobacteriaceae* with reduced susceptibility to carbapenems.

## MATERIAL AND METHODS

The study included 45 strains of *Enterobacteriaceae* with reduced susceptibility to carbapenems, isolated from clinical specimens within one year. These strains were cultured from patients treated at the Dr Antoni Jurasz University Hospital No.1 in Bydgoszcz at the hospital clinics. Isolated strains constituted 4.0% of all of the *Enterobacteriaceae* strains isolated in the given period of time. 24 (53.3%) of the 45 strains with reduced susceptibility to carbapenem were identified as *Klebsiella pneumoniae*, 13 (28.9%) as *Enterobacter cloacae*, 2 (4.5%) as *Citrobacter freundii*, 2 (4.5%) as *Escherichia coli* and four as other species (8.8%) - *Enterobacter cancerogenus*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Raoultella ornithinolytica*. The analysed strains were isolated from: urine - 13 strains (28.9%), blood and bronchoalveolar lavage - six strains

from each (13.3% ), 5 - wound swabs (11.2%) and other clinical materials - 15 (33.3%).

Identification of the strains was carried out based on the results of biochemical reactions performed in identification cards GN (bioMérieux). Reading was performed using an automated system VITEK 2 Compact (bioMérieux). The identification of *R. ornithinolytica* strain was further confirmed using the technique called Matrix-Assisted Laser Desorption Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS) (Bruker, Germany). The assay was done twice to yield four scores: all for *R. ornithinolytica* - 2.39/2.42/2.48/2.49 (medium 2.445). Thus, the obtained score ( $\geq 2.300$ ) indicated the correct identification at the species level. Susceptibility testing to carbapenems and determining Minimal Inhibitory Concentration (MIC) was carried out using E - test (bioMérieux) for ertapenem and imipenem, and agar dilution method for meropenem. The results were interpreted in line with the EUCAST guidelines [9]. In case of detection strains with reduced susceptibility to at least one carbapenem, tests for presence of beta - lactamases types metallo-beta-lactamase (MBL) and *Klebsiella pneumoniae* carbapenemase (KPC) were performed in line with the EUCAST guidelines [10].

## RESULTS

The analysis of the strains with reduced susceptibility to at least one carbapenem showed that the antibiotic which caused the highest susceptibility was meropenem - 42 (93.4%). A high percentage of susceptible strains was also observed in relation to imipenem - 38 (84.5%) strains were considered susceptible. Only 2 (4.5%) strains were susceptible to ertapenem. Seven (15.5%) of analysed strains were moderately sensitive to ertapenem, 5 (11.1%) - to imipenem and 2 - (4.5%) to meropenem. Carbapenem MICs are shown in Table I.

Meropenem exhibited high activity against *Enterobacteriaceae* strains with MICs ranging from 0.125 µg/ml to 32 µg/ml. The MIC for 90% of the investigated strains was the lowest for meropenem and amounted to 0.5 µg/ml, while for ertapenem it was the highest and amounted to 24 µg/ml. Similarly, the MIC for 50% of the tested strains was the lowest for meropenem and imipenem - it amounted to 0.25 µg/ml. MIC<sub>50S</sub> for ertapenem was the highest and amounted to 16µg/ml. MIC<sub>90S</sub> for imipenem amounted to 1 µg/ml.

Tabela I. Wartości MIC karbapenemów dla analizowanych szczepów (n=45)

Table I. Carbapenem MICs for the analysed strains (n = 45)

MICs (µg/ml)	Imipenem	Meropenem	Ertapenem
0.125	3	15	-
0.19	4	-	2
0.25	13	15	-
0.38	2	-	-
0.5	8	5	-
0.75	1	2	4
1	-	2	3
1.5	1	-	-
2	6	3	-
3	2	1	-
4	2	1	2
6	1	-	-
8	1	-	2
32	1	1	32

Apart from the reduced susceptibility to carbapenems in the analysed strains, other resistance mechanisms were also detected. Extended - spectrum beta-lactamases were found in 35 (77.8%) of the investigated strains. In addition, 9 (20.0%) of the strains produced AmpC - beta lactamases. None of the tested strains produced carbapenemases KPC - type and/or MBL. The study showed that 6 (13.4%) of the analysed strains were intermediate or resistant to at least two used carbapenems.

## DISCUSSION

*Enterobacteriaceae* strains with reduced susceptibility to carbapenems are becoming an increasingly important clinical problem worldwide. Analyzing species affiliation of the investigated strains both in our study and the studies of other authors [2, 3, 4]. *K. pneumoniae* rods were isolated most frequently. The percentage of strains of this species isolated in this study and in other researches [2, 3] was over 50%. The second species isolated most frequently was *E. cloacae* - the percentage of strains of this species amounted to almost 29%. Investigators noted 20.4% of strains of *E. cloacae* [2] and 40.2% of strains of *E. cloacae* [3]. The two species isolated during this study and during the studies of researchers from the United States constituted the majority of the investigated strains - over 82% and 95.1% [3]. The lower percentage of strains of the two species was noted in other studies - 47.17% [4] and 73.46% [2]. Similarities in the origin

of the researched strains may be noted. In our study, most strains of the *Enterobacteriaceae* family with reduced susceptibility to the carbapenem were isolated from the urine - almost 29%, then from the blood and respiratory tract - slightly above 13% from each. Similar frequency of occurrence of such a phenotype strains was noted by Americans investigators - 40.2% from the urine, 24.5% from respiratory tract and 16.7% from the blood [3].

Results of own research and the literature cited confirmed that most strains were susceptible to meropenem. This applies to both strains carbapenemase - positive and carbapenemase - negative. In the present study from 45 strains only slightly more than 2% were resistant to that antibiotic. Other researchers noted much higher percentages of strains resistant to meropenem, 32.2% [2], 37.5% [4] and even 74.5 % [3]. The differences in the data may result from antibiotic policy used in the above-mentioned hospitals and from the number of researched strains. It should be emphasized that in the strains investigated in the study of American researchers [3, 4], high resistance to carbapenems was associated with the production of carbapenemases.

Among all of the carbapenems the lowest susceptibility showed ertapenem. It has been confirmed by the results obtained by other authors [2, 3, 4]. The percentage of strains resistant to the drug was 80%. Other authors have noted ertapenem resistance in the range of 95% to 100%. The results of the above-cited works relate to both carbapenemases producing strains as well as to strains with other mechanisms of resistance to those group of drugs [2, 3, 4]. Apart from producing carbapenemases, the susceptibility to carbapenems is significantly influenced by active ejection mechanism of the drug from the cell, called efflux pump and also by deficiency of porin expression [2, 11].

## CONCLUSIONS

The study showed that none of the tested strains produced carbapenemases. In this situation, carbapenems still seem to be an effective therapeutic option in the treatment of severe infections. Despite this, an ever-increasing resistance to this group of antibiotics should not be ignored. The spread of bacterial resistance mechanisms should be counteracted through rational antibiotic therapy and

continuous monitoring of strains in the context of susceptibility and resistance mechanisms.

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