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The investigation of the antibiotic resistance spiral in case of Gram-negative bacteria using time-series analysis

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Introduction

The spread of antibiotic resistance has become one of the most severe problems of modern healthcare in recent years. Presently multiresistance can be observed in the case of many pathogenic bacteria, besides, the spread of panresistant bacteria shows a growing tendency. Following the discovery of penicillin in 1928, development of several other antibiotics commenced, which contributed to the successful treatment of bacterial infections and the rapid decrease of the mortality rate. On the other hand, the inconsequent and improper use of these antibiotics led to the appearance and spread of antibiotic resistant bacteria.

According to the available international reports, more than 2 million infections caused by antibiotic resistant pathogens and 29.000 death cases have been registered in the USA alone in recent years, costing the American healthcare 4,7 billion USD. Similarly, more than 33.000 deaths and an additional cost of more than 1,5 billion USD can be attributed to these infections in Europe.

In the case of Gram-positive bacteria, one of the most frequent problems is caused by MRSA (methicillin-resistant *Staphylococcus aureus*), which shows resistance not only against methicillin but often against aminoglycosides, macrolides, tetracyclines, chloramphenicol and lincosamides as well. Among Gram-negative bacteria, mostly *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* show multi- or even panresistance.

The spread of resistant bacteria has reached such a high level that the World Health Organization (WHO) decided to classify antibiotic resistance as one of the 10 most severe problems threatening humankind. There are multiple factors contributing to the development and spread of antibiotic resistance. These include, among others, the incorrect and increased use of antibiotics, the non-compliance with hygiene rules as well as the lack of appropriate education and neglecting of prevention. Reducing antibiotic resistance is a tremendously complex task requiring the close cooperation of multiple sectors.

Literature Review

The first antibiotics and resistance mechanisms developed against them

The discovery and development of antibiotics is considered one of the greatest achievements of the 20th century. Ever since the discovery of penicillin in 1928, millions of patients suffering from severe bacterial infections have been saved with their help. The period from the 1940s to the 1960s is known as the golden age of antibiotic development, which started with the discovery of streptomycin, and half of the antibiotics used today were discovered during these years. Although the introduction of the most recently discovered antibiotics (linezolid, daptomycin and retapamulin) to therapy took place in the period from 2000 to 2007, their chemical structure and antibiotic effect were known back in the 20th century.

Due to the discovery and routine clinical application of antibiotics, the mortality and morbidity rate started decreasing significantly. In parallel, however, bacteria resistant to antibiotics started spreading quickly. Following the introduction of penicillin to therapy in the 1940s, more than 80 percent of hospital-acquired and ambulatory *Staphylococcus* infections have become penicillin resistant by the end of the 1960s. As a result, the production and use of lactamase resistant penicillins came to the front. The first semi-synthetic, penicillinase-resistant penicillin derivative, methicillin was introduced in 1961, then the first methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated in the UK in 1962 and in the USA in 1968. The production of vancomycin was the first notable success of research on defeating MRSA but after some time, vancomycin resistant bacteria appeared all over the world.

The cephalosporins were introduced to clinical practice in 1964, then they became widespread after the third generation reached the market in the 1980s, and they offered successful therapeutic options against previously known beta-lactamase producing bacteria like *Escherichia coli* producing ampicillin hydrolyzing TEM-1 or *Klebsiella pneumoniae* producing SHV-1-lactamase. However, their excessive

empirical use led to the appearance of bacteria producing Extended Spectrum Beta-Lactamases (ESBLs). As a result, carbapenems gained ground in the empirical therapy for severe infections. Carbapenems showed stability towards most known resistance mechanisms based on beta-lactamase production and have been proved to be effective, among others, in treating infections caused by the ESBL producing *Klebsiella pneumoniae* and *Escherichia coli*. On the other hand, the increasing use of carbapenems resulted in the appearance of the production of carbapenemases providing resistance against them. The first carbapenemase, the IMP-1 was detected in Japan in 1991. In the following years, several other resistance mechanisms started spreading, thus causing a worrisome issue all over the world. Choosing the right antibiotic against carbapenem resistant Gram-negative bacteria is a difficult task, as other beta-lactams as well ss alternatives to beta-lactams, fluoroquinolones and aminoglycosides turned out to be ineffective in most cases. As a result, the application of polymyxins became frontline therapy. Latwer, the beta-lactam antibiotics (ceftolozane-tazobactam, ceftazidime-avibactam and cefiderocol) have been introduced to the therapy for infections caused by these bacteria.

The antibiotic resistance spiral

As a result of the presence of multiresistant pathogens, the failure of empirical antibiotic therapy occurs increasingly frequently, which increases the risk of an unfavorable outcome like protracted illness or death. The spread of these bacteria shifts the empirical choice of antibiotics towards broad-spectrum antibiotics. Consequently, both the rational, evidence-based decisions and the fear of inefficiency play increasingly significant role in choosing antibiotics. The selection pressure exerted by the frequent use of broad-spectrum antibiotics favors the development of resistant bacteria, thus causing the increasing use of broad-spectrum antibiotics. This vicious circle is the antibiotic resistance spiral, whose existence was first mentioned by Carlet and his working party.

The application of cephalosporins is common in the case of hospitalized patients as well as in outpatient care. This leads to the spread of cephalosporin resistance, resulting in the increased application of carbapenems in empirical therapy. After some time, this brought about the appearance and the widespread occurrence of carbapenem resistant bacteria. In response, colistin use increases, followed by the appearance of colistin resistant bacteria. Often, these bacteria show resistance against all antibiotics on the market; thus, they are panresistant. The fear of the inefficiency of antibiotics leads to the preference for broad-spectrum antibiotics, which is the major driver of the antibiotic resistance spiral. Despite that antibiotic resistance became a global issue, the resistant bacteria are distributed unevenly across countries. According to the report published by the European Centre for Disease Prevention and Control (ECDC), antibiotic resistant bacteria tend to occur in a greater proportion in countries where the use of antibiotics is more frequent, e.g. in Southern Europe.

Examining the relationship between antibiotic use and resistance

In recent years, several attempts have been made to analyze the relationship between antibiotic use and resistance using traditional statistical methods, mostly linear regression and correlation analysis. However, the resistance data detected in a given month are influenced by the development of antibiotic resistance in the previous months, thus the data points in these data series are not independent. As a result, the data above violate the precondition relating to the independency of data points. Consequently, the aforementioned traditional statistical methods like linear regression are not suitable for the examination and correct interpretation of these correlations.

In order to precisely analyze the relationship between antibiotic use and resistance, time series analysis can be applied. Initially, time series analysis was used in the field of economics to examine stochastic processes. The autoregressive integrated moving average model (ARIMA) was developed by Box and Jenkins in 1976, which provided a means to examine the behavior of a variable over time and to analyze its abrupt changes. Time series are collections of time-varying data measured at regular

intervals. The frequency of regularly recorded data points can be daily, weekly, monthly etc. Contrary to the traditional methods, time series analysis considers the possible correlations between the examined data as well as the change of time series over time and the correlations between them.

Dynamic regression

Dynamic regression makes it possible to examine the relationship between dependent and independent variables as well as the strength of the correlation between them. The advantage of this method is that it allows for examining how multiple independent variables affect the same dependent variable together. Contrary to linear regression, the time series of the independent variable appears in multiple variations in the examined model. The model contains the original time series starting at time zero as well as different versions of it obtained by shifting it backward in time. Contrary to linear regression, both the dependent variable and its lagged values can be used in dynamic regression models, in this manner, lagged effect can also be detected. Since the dependent and independent variables (cause and effect) are predefined in the case of dynamic regression, the reciprocal analysis of these correlations is not possible.

Vector autoregressive model

Vector autoregressive models (VAR) permits the examination of all variables at the same time and description of potential bidirectional relationships. In these models, each examined time series is a linear function of the lagged values of itself and the other variables. Compared to other regression models, VAR models allow for examining the relationships in cases where two or more time series influence each other simultaneously. These models give the opportunity to precisely analyze the relationship between antibiotic use and resistance as well as to predict its development over time. Putting the results into clinical practice might help to rationalize antibiotic use and to reduce the spread of resistance.

Objectives

1. To examine the relationship between antibiotic use and resistance using suitable statistical methods. To analyze the reciprocal effect of the correlations.
2. To examine the assumed resistance spiral in the case of Gram-negative bacteria, highlighting the bacteria determining the steps of the resistance spiral.
3. To compare the results of dynamic regression and VAR models.
4. To examine the evolution of the resistance spiral over time from 2015 to 2019.

Materials and Methods

We gathered and analyzed data on antibiotic use and resistance from the University of Debrecen with 1667 beds. The study covered the period from October 2004 to December 2019.

We collected data on the consumption of all antibacterials classified in ATC group J01 (antibacterials for systemic use) broken down by month. We expressed antibiotic consumption by the number of defined daily doses (DDD) calculated on the basis of the recommendation issued by WHO, which we standardized for 100 patient days. We analyzed the consumption of carbapenems, aminoglycosides, fluoroquinolones, colistin as well as cephalosporins, including 3rd generation cephalosporins separately.

In parallel, we followed the incidence density of infections caused by the cephalosporin resistant *Escherichia coli*, the 3rd generation cephalosporin resistant *Klebsiella spp.* (*K. pneumoniae*, *K. oxytoca* together), the ceftazidime resistant *Pseudomonas aeruginosa*, the carbapenem resistant *Escherichia coli,* the carbapenem resistant *Klebsiella spp.* (*K. pneumoniae*, *K. oxytoca* together), the carbapenem resistant *Pseudomonas aeruginosa* and the carbapenem resistant *Acinetobacter baumannii* per 1000 patient days broken down by month. In case a patient showed multiple positive

results, we took into account only the first sample. The group of 3rd generation cephalosporin resistant Gram-negative bacteria was created from the data on the 3rd generation cephalosporin resistant *Escherichia coli*, the 3rd generation cephalosporin resistant *Klebsiella spp.* (*K. pneumoniae*, *K. oxytoca* together) and the ceftazidime resistant *Pseudomonas aeruginosa*. The group of carbapenem resistant Gram-negative bacteria was cumulated as the sum of the carbapenem resistant *Klebsiella spp.* (*K. pneumoniae, K. oxytoca* together), the carbapenem resistant *Pseudomonas aeruginosa* and the carbapenem resistant *Acinetobacter baumannii*. In the case of *Acinetobacter baumannii*, the examination of cephalosporin resistance is not recommended by EUCAST, therefore it was omitted from the analysis.

These data draw a hypothetical antibiotic resistance spiral: cephalosporin use – cephalosporin resistance, cephalosporin resistance – carbapenem use, carbapenem use – carbapenem resistance, carbapenem resistance – colistin use, colistin use – colistin resistance.

During the first analysis, we analyzed the data from the period from October 2004 to August 2016 along this assumed resistance spiral. First, we observed all listed Gram-negative bacteria collectively, then we picked species one by one to examine the relationship between antibiotic use and resistance. In the first half of the examination, we examined antibiotic use – resistance and resistance – antibiotic use data pairs. In the case of the simplest models, we examined how the variables affected each other in pairs. After that, we created new models by supplementing the original ones with variables representing aminoglycoside and fluoroquinolone consumption in order to build models focusing on their effect.

We applied two methods to carry out time series analysis. We tested the effect of values collected 0-6 months prior (lag) using dynamic regression models, consisting of either simply two variables or with additional variables representing aminoglycoside and fluoroquinolone consumption. We created the models in Eviews 3.1 (Quantitative Micro Software, Irvine, CA). While building the models, we considered the principles described by Pankratz as well as López-Lozano et al. The appropriate model was

achieved by eliminating the insignificant lags step by step. Model diagnostics included testing the normality of residuals (Jarque - Bera test) and the autocorrelation between them (Ljung - Box test, Breusch – Godfrey test). The model was considered appropriate when the residuals showed normal distribution and no autocorrelation was observed.

We built the VAR models with the help of the R software environment for statistical computing, including the vars, seastests and fUnitRoots packages. First, we examined the presence of seasonality (yearly), trend or unit root for each variable using the Dickey-Fuller test. Whenever we observed the presence of trend or seasonality for a variable, we controlled for the variable by adding a trend and/or seasonality variable to the model. Choosing the optimal lag was based on the Akaike information criterion. In case the recommended lag resulted in a wrong model, we accepted the smallest lag leading to a valid model. Model diagnostics and dynamic regression were conducted similarly. In the case of VAR models, we examined the correlation between pairs of variables using impulse response functions, which examine the cause-and-effect relationship between two variables (impulse variable and response variable) picked from the model. After shocking the system by incrementing the impulse variable by one unit, we observe how the response variable changes in a given time interval, which spanned 12 months in our case. We calculated the 95% confidence intervals using bootstrapping. By performing 100 consecutive examinations, we can determine the interval which includes 95 of the 100 measurements. In case the zero value was not within this interval, we can interpret this as a significant change in the response variable.

Since the models were sensitive to the fluctuation of the last values of the time series, we applied a rolling window strategy to examine the stability of the models over time. This was performed by creating 12 separate models from the same variable set by shortening the time series by a month each time. The correlation between the variables was considered acceptable when we detected the significant correlation at least in 6 of the 12 cases.

The process of building VAR models resembled the process of building dynamic regression models. We created models made up of two variables for each pair

of variables as well as models containing additional variables representing aminoglycoside and fluoroquinolone consumption. Finally, we built models containing all variables. After analyzing the data considering Gram-negative bacteria as a group, we analyzed the data broken down by species, then we created the model of the whole antibiotic resistance spiral by grouping together the significant variables in the same model.

During the second analysis, we created shortened time series by dropping the last 12 months from the time series, thus, we worked with the complete time series (October 2004 – December 2019) as well as with four additional shorter time series (October 2004 – December 2018, October 2004 – December 2017, October 2004 – December 2016, October 2004 – December 2015). This examination focused entirely on building VAR models, which were created for each time series as described above. We made a year-to-year comparison between the obtained correlations based on the impulse-response functions.

Results

The antibiotic consumption from October 2004 to August 2016

During the examination period, antibiotic use showed a growing tendency in the case of all examined groups of antibiotics. The total cephalosporin use increased from 4.57 DDD/100 patient days to 10.5 DDD/100 patient days. By August 2016, the carbapenem consumption has reached 4.32 DDD/100 patient days, starting from 0.79 DDD/100 patient days. During the period of study, colistin was used for the first time in January 2007, followed by June 2007, then its use has become fairly continuous from 2009.

Antibiotic resistance from October 2004 to August 2016

We observed a growing tendency while examining the frequency of the appearance of antibiotic resistant bacteria. It is important to highlight the change in the

number of carbapenem resistant bacteria: by 2016, the resistance developed against carbapenems has shown a growing tendency in the case of all Gram-negative isolates as well as *Acinetobacter baumannii*. Besides, the number of cephalosporin resistant *Escherichia coli* and *Klebisella spp.* isolates increased as well. During the examination period, colistin resistant *Acinetobacter baumannii* bacteria were found for the first time in August 2009. Subsequently, resistant *Acinetobacter baumannii* bacteria were isolated on multiple occasions each year except 2012, the highest incidence density of 0.055/1000 patient days was observed in December 2015.

The resistance spiral in the case of Gram-negative bacteria

First, we examined the relationship between antibiotic use and resistance pairwise, then we built models which considered the effect of aminoglycosides and fluoroquinolones as well. Both dynamic regression and bivariate VAR models showed a significant relationship between the use of 3rd generation cephalosporins and cephalosporin resistance. The models which we used to analyze how the consumption of all cephalosporins affects resistance showed the same result. Neither in the case of bivariate VAR models, nor in the case of models considering other antibiotic groups had cephalosporin resistance an effect on the consumption of 3rd generation cephalosporins. The increase in the number of cephalosporin resistant Gram-negative bacteria contributed to the growing use of carbapenems significantly. On the other hand, the increased carbapenem use did not decrease the appearance of cephalosporin resistant bacteria significantly according to the models. A significant relationship was detected between carbapenem use and resistance in the case of dynamic regression as well as both VAR models, none of the models showed reciprocal effects. We detected a significant, basically instantaneously visible relationship between carbapenem resistance and colistin use in both types of models. Besides, the reciprocal effect of colistin use on carbapenem resistance was visible in the VAR models. The relationship between colistin use and resistance showed only a weak correlation in dynamic

regression models, however, it was not present at all in either the bivariate or the multivariate VAR model.

The next step involved building a model containing all previous variables; thus, all elements of the entire resistance spiral. Whether we considered the use of all cephalosporins or only the use of 3rd generation cephalosporins, the previously observed relationship between cephalosporin use and resistance was not detected. Although the relationship between carbapenem use and resistance remained, it was weaker compared to what we observed in pairwise models. Contrary to this, the correlation between antibiotic resistance and the new antibiotic group, whose usage is caused by antibiotic resistance, proved to be strong, therefore, the correlation between cephalosporin resistance – carbapenem use and carbapenem resistance – colistin use was significant.

Examining the relationship between antibiotic use and resistance by species

Escherichia coli

While examining the relationship between antibiotic use and resistance in the case of *Escherichia coli*, we found a significant correlation between cephalosporin use and cephalosporin resistance as well as between cephalosporin resistance and carbapenem use, which was present in dynamic regression models as well as bivariate and multivariate VAR models. Since the sample size of the carbapenem resistant *Escherichia coli* bacteria was small during the examination period, we did not examine either the effect of carbapenem use on carbapenem resistance or the effect of the latter on colistin use.

Klebsiella spp.

In the case of *Klebsiella spp.*, dynamic regression showed way weaker relationships between cephalosporin use and resistance, cephalosporin resistance and carbapenem use as well as carbapenem use and resistance. None of these relationships proved to be significant in the bivariate VAR models, while in the multivariate VAR

models, the relationship was detected only between cephalosporin use and resistance. The carbapenem resistance of *Klebsiella spp.* did not contribute to the significant increase in colistin consumption in any case.

Pseudomonas aeruginosa

In the case of *Pseudomonas aeruginosa*, a significant relationship was detected between cephalosporin resistance and carbapenem use as well as carbapenem use and carbapenem resistance. The relationship between carbapenem use and resistance appeared in bivariate VAR models with 2 lags as well as in multivariate models with 1 lag or 2 lags. Carbapenem resistance did not influence colistin use in any model.

Acinetobacter baumannii

In the case of *Acinetobacter baumannii*, carbapenem use contributed to the increasing number of carbapenem resistant bacteria, which induced the increase in colistin use. These relationships were present in dynamic regression models as well as in bivariate and multivariate VAR models. The significant relationship between carbapenem use and carbapenem resistance was detected in the bivariate VAR model with a lag of 1 month and in the multivariate model with a lag of 1-4 months.

The antibiotic resistance spiral at the University of Debrecen in the case of Gram-negative bacteria

The relationship between cephalosporin use and resistance detected earlier was not shown. However, the increasing number of the cephalosporin resistant *Escherichia coli* bacteria provoked the use of carbapenems. The carbapenem consumption brought with it the upsurge in the number of carbapenem resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. The carbapenem resistant *Acinetobacter baumannii* shifted the spiral towards colistin use.

The evolution of the resistance spiral in the case of Gram-negative bacteria

Cephalosporin use increased cephalosporin resistance in the case of all 5 time series (2015-2019), as a result, carbapenem use increased. The spread of carbapenem resistant pathogens was facilitated by the growth of carbapenem use, thus increasing colistin use. Colistin resistance was not affected by colistin use in the case of any time series. These correlations were present in the case of each time series we examined (2015, 2016, 2017, 2018, 2019). The effect of cephalosporin use on carbapenem resistance was weaker in the case of the time series ending in 2018. The effect of carbapenem use on carbapenem resistance started being highly significant in 2017.

The evolution of the resistance spiral by species

Escherichia coli

In the case of Escherichia coli, the time series showed a significant correlation between cephalosporin use and the resistance against 3rd generation cephalosporins each year except 2019. The effect of cephalosporin resistance on carbapenem use was present in 2015 and 2016.

Klebsiella spp.

In the case of *Klebsiella spp.*, only the time series ending in 2015 and 2019 showed the relationship between cephalosporin use and the resistance against 3rd generation cephalosporins. The correlation between the increasing number of the cephalosporin resistant *Klebsiella* bacteria and the growth of carbapenem resistance was observed in 2018, although it was weak. No relationship was observed between carbapenem use and carbapenem resistance in any year.

Pseudomonas aeruginosa

A weak relationship was observed between carbapenem use and the increasing number of the carbapenem resistant *Pseudomonas aeruginosa* in the case of the time

series ending in 2016. Furthermore, carbapenem resistance contributed to the significant growth of colistin use in the case of the time series ending in 2015 and 2016.

Acinetobacter baumannii

The occurrence of the carbapenem resistant *Acinetobacter baumannii* was significantly increased by carbapenem use in the case of each time series we studied. This, in parallel, this shifted the spiral towards the growth of colistin use.

The evolution of the antibiotic resistance spiral in the model containing all variables

Neither *Escherichia coli*, nor *Klebsiella spp.* showed a significant relationship between cephalosporin use and cephalosporin resistance in any year. In the case of the time series ending in 2015 and 2016, we observed that the increase in carbapenem use was strongly influenced by the occurrence of the cephalosporin resistant *Escherichia coli*. In 2017, this influence was weak but significant. Since 2017, carbapenem use led to an increase in the number of the carbapenem resistant *Acinetobacter baumannii*. In 2016, we detected that the carbapenem resistant *Pseudomonas aeruginosa* influenced positively the growth of colistin use. The relationship between the carbapenem resistant *Acinetobacter baumannii* bacteria and colistin use was observed each year.

Discussion

The spread of multiresistant bacteria is one of the greatest challenges of healthcare in the 21st century. The burden of disease and the excess mortality caused by multiresistant pathogens is measurable, more than 33.000 death cases are associated with infections caused by these bacteria in the EU each year. In recent years, the fear of resistance has made carbapenems first-line antibiotics in parallel to the spread of ESBL-producing bacteria. The spread of antibiotic resistance shifted antibiotic use towards broad-spectrum antibiotics, which resulted in the appearance and spread of more

resistant bacteria, especially among Gram-negatives. This resistance spiral was clearly visible in consecutive dynamic regressions and VAR models.

Based on our examinations, cephalosporin use led to the spread of the cephalosporin resistant *Escherichia coli*, this relationship can be observed internationally as well. International data shows an increase in cephalosporin use in the case of hospitalized patients as well as in outpatient care. This way, the spread of ESBL-producing bacteria can be explained by the increasing use of cephalosporins, which is fully consistent with the results of the present work. Although the resistance against this antibiotic group shows a growing tendency, the negative effect of cephalosporin resistant bacteria on cephalosporin consumption was not detected in our models, therefore, the consumption of the antibiotic group does not decrease despite the spread of cephalosporin resistance, consequently the increasing application of cephalosporins is the result of their empirical prescription.

The VAR models allows for examining the relationships the other way around, therefore, it is possible to examine how the spread of cephalosporin resistant bacteria affects cephalosporin use. Although it can be assumed that the spread of resistant bacteria and thus the decrease in the expected effectiveness of cephalosporins reduce cephalosporin use, the results of the models suggest that cephalosporins are still used frequently despite the spread of cephalosporin resistance. Therefore, cephalosporins were not replaced by carbapenems, but carbapenems were used in addition to cephalosporin consumption.

The spread of cephalosporin resistant bacteria favors the increase in carbapenem use. The use of carbapenems increases the occurrence of carbapenem resistance in the case of all four important Gram-negative hospital pathogens. In our present study, the proportion of carbapenem resistance remained low in the case of *Escherichia coli* and *Klebsiella spp*., the relationship between carbapenem consumption and resistance was emphasized primarily in the case of *Acinetobacter baumannii*. This data is consistent with the molecular epidemiological studies on the carbapenem resistant *Acinetobacter baumannii* bacteria conducted by the working group. Based on

the results of these studies, carbapenem use and its pattern influenced the spread of *Acinetobacter baumannii* and the competition between them, besides, the dominance of meropenem use might have played a role in the spread of the *Acinetobacter baumannii* ST636 bacterium. This effect was always shown in all lagged time series as well, therefore, it can be considered constant through the entire examination period from 2015 to 2019.

Against carbapenem resistant isolates, colistin is one of the last potentially effective antibiotics. Considering that primarily *Acinetobacter baumannii* was responsible for carbapenem resistance in the antibiotic resistance spiral, it is not surprising that it had the strongest and most constant relationship with the increasing colistin use as well.

Probably due to the low incidence rate of colistin resistance, no statistical relationship was detected between colistin use and resistance during the study period. Based on the observations from previous years, the rising incidence rate of the colistin resistant *Acinetobacter baumannii* and other Gram-negative bacteria can be expected in the upcoming period.

When examining the evolution of the resistance spiral on the time series analyzed year by year separately, the relationship between antibiotic use and the resistance changes dynamically depending on the structure of the time series. In certain years, the relationships can be weaker, occasionally, they can even disappear. The dominant role of *Acinetobacter baumannii* compared to other Gram-negative bacteria must be highlighted, since the significant relationship between carbapenem use and carbapenem resistance was detected in the case of each time series. Carbapenem resistant *Acinetobacter baumannii* plays an important role in the increase in the mortality and morbidity rate at an international level. As a result, in 2017, carbapenem resistant *Acinetobacter baumannii* was classified by WHO as one of the pathogens against which there is an urgent need to develop new antibiotics. The pandemic increased the importance of the infections caused by carbapenem resistant *Acinetobacter baumannii* even further, the number of infections caused by resistant

bacteria showed a growing tendency, which might have been facilitated by the routine prophylactic and empirical application of broad-spectrum antibiotics, including carbapenems.

Although the dynamic regression and VAR models used to analyze the relationship between antibiotic use and resistance gave mostly similar results, in the case of VAR models the relationships were not always detected because of the stricter model acceptance criteria.

According to our examination, different pathogens play an important role on each step of the resistance spiral. While cephalosporin use causes the spread of the cephalosporin resistant *Klebsiella spp.* and *Escherichia coli*, the increase in carbapenem use favors the appearance of the carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. As carbapenem resistance was especially rare in the case of *Klebsiella spp.* and *Escherichia coli*, consequently, the effect of carbapenem use was detected in the spiral mainly in the case of *Acinetobacter baumannii*.

The antibiotic stewardship activity often requires forced prioritization and focus on solving particular problems, therefore, the methods set out in the present work can act as a tool to plan it in an evidence-based manner.

Summary

The discovery of antibiotics in the 20th century and the ever-increasing rate of their application saved millions of patients suffering from bacterial infections, thus becoming one of the most significant medical innovations. On the other hand, the excessive and inappropriate use of these antibiotics led to the rapid spread of antibiotic resistance which contributed substantially to the increasing growth of the mortality and morbidity rate. Examining the relationship between antibiotic use and resistance as well as making predictions play an ever more important role in reducing antibiotic resistance.

In order to analyze the relationship between antibiotic use and resistance, we used statistical methods which allowed for following the correlation between the variables and to observe their dynamics and development over time. Furthermore, we analyzed the assumed antibiotic resistance spiral and determined the bacteria maintaining it. We examined these relationships with the help of dynamic regression and vector autoregressive models. Although both statistical methods showed similar results while analyzing the data, we detected less correlation between the variables using VAR models because of the stricter conditions of model building and acceptance.

A strong correlation could be observed between antibiotic use and resistance. The spread of cephalosporin resistant Gram-negative bacteria was facilitated by the increasing use of cephalosporins. The reaction of healthcare to this was the empiric prescription of carbapenems. The growing carbapenem use brought about the appearance and spread of carbapenem resistant bacteria and consequently, colistin use gained ground.

Different species play different role at each step of the antibiotic resistance spiral. As a result, the increasing use of cephalosporins contributed to the spread of cephalosporin resistant *Klebsiella spp.* and *Escherichia coli.* The cephalosporin resistant *Escherichia coli* provoked the growth of carbapenem use which led to the carbapenem resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* gaining ground. The carbapenem resistant *Acinetobacter baumannii* significantly influenced the increase in colistin use. Based on the examination of the connection between antibiotic use and resistance using shortened time series, the relationships proved to be dynamic,

Containing antibiotic resistance is one of the most important tasks in the field of medicine in the 21th century. The examination of antibiotic use and resistance using statistical methods allows for examination and prediction of these relationships, The results can contribute to the optimization of the current antibiotic prescription habits which can slow down and, hopefully, may reduce the spread of resistant bacteria.

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Candidate: Hajnalka Tóth
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List of publications related to the dissertation

1. **Tóth, H.**, Buchholcz, G., Fésüs, A., Balázs, B., Nagy, J. B., Majoros, L., Szarka, K., Kardos, G.: Evolution of the Gram-Negative Antibiotic Resistance Spiral over Time: a Time-Series Analysis.
   Antibiotics-Basel. 10 (6), 1-10, 2021.
   DOI: http://dx.doi.org/10.3390/antibiotics10060734
   IF: 5.222

2. **Tóth, H.**, Fésüs, A., Kungler-Gorácz, O., Balázs, B., Majoros, L., Szarka, K., Kardos, G.: Utilization of vector autoregressive and linear transfer models to follow up the antibiotic resistance spiral in Gram-negative bacteria from cephalosporin consumption to colistin resistance.
   Clin. Infect. Dis. 69 (8), 1410-1421, 2019.
   DOI: http://dx.doi.org/10.1093/cid/ciy1086
   IF: 8.313

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List of other publications

3. Balázs, B., Tóth, Z., Nagy, F., Kovács, R. L., **Tóth, H.**, Nagy, J. B., Tóth, Á., Szarka, K., Majoros, L., Kardos, G.: The Role of Uniform Meropenem Usage in Acinetobacter baumannii Clone Replacement.
Antibiotics. 10 (2), 1-12, 2021.
DOI: http://dx.doi.org/10.3390/antibiotics10020127
IF: 5.222

Total IF of journals (all publications): 18,757
Total IF of journals (publications related to the dissertation): 13,535

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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