

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**RELATIONSHIP BETWEEN ANTIBIOTIC CONSUMPTION AND
EPIDEMIOLOGY OF RESISTANCE MECHANISMS AMONG NON-
FERMENTATIVE NOSOCOMIAL PATHOGENS**

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The Examination takes place at the Department of Pharmacology, Faculty of Medicine,
University of Debrecen, at 11:00 a.m. on 21st of May, 2015

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal
Medicine, Faculty of Medicine, University of Debrecen, at 1:00 p.m. on 21st of May, 2015.

INTRODUCTION

Previously, the multidrug-resistant Gram-positive bacteria (for example methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin moderately susceptible *Staphylococcus aureus* (VISA), penicillin-resistant *Streptococcus pneumoniae*) had been reported to be the most important and most difficult problems, but presently the multidrug-resistant Gram-negative bacteria such as multidrug-resistant *Pseudomonas aeruginosa*, multidrug-resistant *Acinetobacter baumannii* (MACI), extended-spectrum β -lactamase (ESBL) producing enterobacteria have become the most important hospital pathogens.

P. aeruginosa and *A. baumannii*, as opportunistic microorganisms, often cause life-threatening infections, exploiting local or systemic disorders of the immune-system. Twenty percent of all nosocomial infections is caused by *P. aeruginosa* and 2-10% by *A. baumannii*. Ventilator-associated pneumonia (VAP) generally occurs within 48-72 hours after endotracheal intubation and its lethality could be as high as 70%. Besides VAP, bloodstream infections (34%), catheter-associated urinary tract infections (40%), skin and surgical-site infections are the most significant infections caused by these bacteria. Secondary meningitis caused by *A. baumannii* develops after neurosurgery operations and has a similar lethality ratio (70%) to VAP.

Due to multiple resistance to beta-lactam antibiotics, the application of other antibiotics have become more important, particularly the use of aminoglycosides and fluoroquinolones, as well as of other groups of antibiotics such as tigecyclin and polymyxins, the latter two remaining effective against multidrug-resistant pathogens. As a result, the use of these antibiotics is increasing, which consequently led to increase of the resistance rates against them.

The resistance to aminoglycosides is caused by a number of mechanisms including enzymatic modification, decreased membrane permeability, methylation of 16S rRNA, mutations in the 16S rRNA or in certain ribosomal proteins and active efflux. In carbapenem resistance the major mechanisms are β -lactamase production, porin loss or active efflux as well as alterations in the target penicillin-binding proteins (PBPs). These mechanisms alone or in combinations result in a high level of resistance to aminoglycosides and carbapenems.

Based on the antibiotic resistance rates published, Hungary rather belongs to the critical areas of Europe. Prevalences of aminoglycoside-resistant invasive *Klebsiella pneumoniae* and *P. aeruginosa* isolates are between 25-50%, while this ratio exceeds 50% in case of *A.*

baumannii. Similar prevalence rates are found in carbapenem resistant *P. aeruginosa*, carbapenem resistant *A. baumannii* and fluoroquinolone resistant *P. aeruginosa*. According to data of the 2013 Monitoring System, 1.9% of isolates belonging to *A. baumannii-calcoaceticus* complex possess MIC values above 4 mg/l against colistin.

Mobile genetic elements (plasmids, transposons, integrons) play an important role in the spread of antibiotic resistance, and accelerate the spread of acquired resistance significantly among different species of bacteria by horizontal gene transfer. The selection of multidrug-resistant and extensively drug resistant strains led to serious problems in the health care system, because the therapeutic options have been narrowed and only tigecycline and colistin as a last antibiotic resort could be effective against them.

AIMS

The aim of this study was to investigate the relationship between the consumption of carbapenems and aminoglycosides and the occurrence of resistance to antimicrobial agents at different intensive care units of the University of Debrecen in the case of *P. aeruginosa* and *A. baumannii*.

1. Examination of the genetic background of the aminoglycoside resistance mechanisms among *P. aeruginosa* and *A. baumannii* isolates.
2. Examination of genetic background of carbapenem resistance mechanisms among *A. baumannii* isolates.
3. Examination of relationship between integron carriage and resistance.
4. Analysis of genetic relatedness of the isolates by pulsed-field gel electrophoresis.
5. Examination of the relationship between carriage of resistance genes and clonality
6. Investigation of the connection between consumption of aminoglycoside antibiotics in the Pulmology Clinic and the aminoglycoside resistance of *P. aeruginosa* clones.
7. Investigation of the connection between consumption of carbapenem antibiotics and the emergence of carbapenem resistance in *A. baumannii* clones

MATERIAL AND METHODS

Isolates

Altogether 98 *P. aeruginosa* isolates (isolated between December 2008 and February 2010 from the Pulmonology Clinic) and 160 *A. baumannii* isolates (collected between November 2010 and May 2011 from different intensive care units, which represents 46.9% of patients infected with *A. baumannii* during the study period) were collected from clinics of the University of Debrecen. The majority of the *P. aeruginosa* isolates were obtained from lower airway samples (bronchial washing, tracheal aspirate, sputum) and most *A. baumannii* isolates were isolated from bronchial, blood, canule, wound and tube samples. The isolates were identified based on their biochemical properties; species identification was confirmed by PCR specific for *P. aeruginosa* and in case of *A. baumannii* by MALDI-TOF mass spectrometry.

Antibiotic susceptibility was determined by the CLSI (Clinical and Laboratory Standards Institute, 2010) disk diffusion method. The following antibiotics were tested, imipenem, meropenem, piperacillin+tazobactam, ceftazidime, cefepime, ciprofloxacin, amikacin, gentamicin, and tobramycin. In addition, colistin, sulfamethoxazole+trimethoprim, doxycycline and tigecycline were also examined in case of *A. baumannii*.

DNA was extracted by heat-treatment at 98 ° C for 15 minutes.

Tested genes

Three aminoglycoside modifying enzyme genes *aac(6')-Ib*, *aac(3'')-Ia*, *ant(2')-IIa* were tested in case of *P. aeruginosa* isolates, whereas six aminoglycoside modifying enzyme genes *aac(6')-Ib*, *aac(3'')-Ia*, *ant(2')-IIa*, *ant(3'')-Ia*, *aph(3')-Ia*, *aph(3')-VIa* were sought for in *A. baumannii* isolates. Furthermore, presence of *armA*, *rmtA*, and *rmtB* genes coding for aminoglycoside resistance methylases was also tested for both species.

Presence of five genes belonging to the carbapenem-hydrolyzing oxacillinase (Ambler D group), *bla*_{OXA-23-like}, *bla*_{OXA-24-like}, *bla*_{OXA-48-like}, *bla*_{OXA-51-like}, *bla*_{OXA-58-like}, were examined in case of *A. baumannii*. In addition, the presence of the *ISAbal-1* insertion sequence was also sought for.

Eighteen genes, which play an important role in the pathogenesis of infections caused by *P. aeruginosa*, were examined; exotoxin gene *toxA*, *algD* gene involved in alginate synthesis, genes encoding effector proteins of the type III. secretion system *exoT*, *exoS*, *exoU*, *exoY*, *plcH*, *plcN* genes encoding phospholipases, *apr*, *lasB* genes coding for proteases, *pilA* gene encoding type IV. pili, operons as well as genes *phzI*, *phzII*, *phzS*, *phzM* involved in phenazine synthesis and pyoverdine receptor genes *fpvA-I*, *fpvA-II*, *fpvA-III*, which act as part of the iron uptake system.

Detection of integrons

Occurrence of resistance integrons was studied using PCRs specific for the class I, II, and III. integrases. Variable regions of the carried integrons were sequenced using the dideoxy chain termination method. The software CLC DNA Workbench was used to assemble and analyse sequences; genes were identified by GenBank (BLAST[®] www.ncbi.nih.gov/blast) searches. Identity of integrons with variable regions of the same size was confirmed by restriction fragment length polymorphism analysis using XbaI and XhoI for *P. aeruginosa*, and XbaI, HindIII and DdeI for *A. baumannii*.

Determination of genotype

Genetic relatedness among isolates was analysed by pulsed-field gel electrophoresis (PFGE). Restriction analysis was performed by 10U restriction enzymes BcuI (SpeI) and ApaI for *P. aeruginosa* and *A. baumannii*, respectively.

DNA banding patterns were analysed with the Fingerprinting II software using the Dice coefficient and the unweighted pair group method with averages (UPGMA).

Antibiotic consumption

The crude antibiotics consumption data was provided by the Central Pharmacy of the University of Debrecen. Based on the WHO recommendation, consumption of antibiotics was expressed in the number of defined daily doses (DDDs) per 100 bed-days or with respect to individual patients using the MS Excel application ABC Calc Version 3.0.

Statistical analysis

P. aeruginosa

The differences in total consumption of antibiotics as well as in aminoglycoside consumption between patients were analysed by Kruskal-Wallis tests and posthoc Mann-Whitney pairwise comparisons with Bonferroni correction.

A. baumannii

Relationship between antibiotic consumption and changes in isolation frequency as well as antibiotic resistance of *Acinetobacter* spp. was analysed by linear regression as a screening method. In case of antibiotics where the consumption data showed significant correlation with prevalence or resistance data, after trend removal cross-correlation analysis was performed using quarterly time lags. Prevalences of carbapenem susceptible and resistant *A. baumannii* at different wards were compared using chi-square test or by means of Fisher's exact test, as appropriate. Consumption of different antibiotic classes at

different wards was compared by means of Kruskal-Wallis test. All statistical tests were performed using PAST 3.0.

RESULTS

P. aeruginosa

Presence of aminoglycoside resistance genes and integrons

The most frequent gene was *aac(6')-Ib* among *P. aeruginosa* isolates. This gene, which confers resistance to amikacin and tobramycin, was detected in 62.2% (61/98) of the isolates. Beside the *aac(6')-Ib* gene, *ant(2'')-Ia* gene coding for gentamicin and tobramycin resistance was found in 16 isolates (16.3%). Another examined genes such as *aac(3')-IIa*, *armA*, *rmtA*, and *rmtB* were never found. The majority of the isolates (92.0%) carried class I. integrons; class II. and III. integrons could be never detected.

Determination of the genotype

Among the 98 isolates tested, three main similarity clusters were identified; further 22 isolates had unique patterns.

Cluster A included 49 isolates from 12 patients. Transmission of a strain with a patient transferred between the wards Pulmonolgy ICU and Pulmonolgy Rehabilitation was also detected. Antibiotic susceptibility of the isolates within the cluster was highly variable. While all isolates were uniformly resistant to ciprofloxacin, amikacin, gentamicin, and tobramycin, resistance to beta-lactam agents were diverse. All isolates harboured a class I integron with a gene cassette array *aac(6')-Ib*, *ant(3')-Ia* and a *bla_{PSE-1}* gene coding for a beta-lactamase (In99). The cluster was characterized by the presence of ferripyoverdin receptor type III and lack of *pilA* and *exoU* genes; all other virulence genes were found in this cluster.

Cluster B included 16 isolates carrying a nucleotidyl transferase gene *ant(2'')-Ia* in the variable region of the integron (In159). Antibiotic susceptibility was characterized by ciprofloxacin, gentamicin, and tobramycin resistance, but resistance to beta-lactams was less extended as compared to cluster A; only five isolates were resistant to antipseudomonal beta-lactam agents. Virulence gene composition was similar to cluster A, except for the presence of ferripyoverdin receptor type I instead of type III.

Cluster C included eight isolates from six patients. One isolate was obtained from a patient in the pulmonology ward (potential importation/exportation). Besides imipenem resistance, three isolates showed variable resistance to beta-lactams, ciprofloxacin, and aminoglycosides. One of these isolates carried a class I integron without gene cassettes (In0). Other aminoglycoside resistance genes were not detected in the cluster. Except *exoS*, all virulence genes sought for were present.

The number of the independent isolates was 22, which originated from 20 patients. Out of the 22 isolates, nine were susceptible to all antipseudomonas antibiotics tested, and only one harboured aminoglycoside resistance genes, i.e. an *aac(6')-Ib* gene. A single isolate harboured an empty integron (In0). Virulence gene distribution was very heterogeneous, only *phzII*, *apr*, *exoT*, and *plcH* genes were present in all isolates. However, the other genes, except *pilA* and *exoU*, were found in the majority (16/22) of isolates. A single isolate harboured all 15 tested virulence determinants.

Antibiotic consumption

Antibiotic consumption of the pulmonology clinic between 2005 and 2009 was above the university average (97.8 versus 38.8 DDDs/ 100 bed-days). Amikacin (2.9 DDD/100 bed-days), gentamicin (2.8 DDD/100 bed-days) and tobramycin (1,4 DDD/100 bed-days) were the most commonly used aminoglycoside agents. Streptomycin and netilmicin

consumption showed a lower average (0.3 and 0.02 DDD/100 bed-days) and were used only occasionally. Between 2006 and 2007, the clinic switched from tobramycin to gentamicin and then partial replacement of gentamicin by amikacin in 2008–2009 was observed. Between 2008 and 2010, 19 of the 37 patients received amikacin; out of these patients 16 were admitted to the ICU and the remaining three patients were found in non-ICU wards of the Department. Gentamicin was administered to three patients; one patient received streptomycin and another one was treated with tobramycin. Consumption of fluoroquinolones was also significant and showed a similar increase (from 16.6 DDD/100 bed-days to 20.7 DDD/100 bed-days) to the aminoglycosides, but a marked decrease with the lowest value observed, in 2009 (12.3 DDD/100 bed-days). Usage of broad-spectrum beta-lactams (piperacillin+tazobactam, carbapenems) was also very frequent during the study period. Only a single patient from the study was treated without antibiotics.

Statistical analysis demonstrated that patients harbouring isolates belonging to cluster A received significantly more amikacin than patients harbouring isolates from other clusters or isolates with unique fingerprints ($P = 0.046$). As overall antibiotic consumption (as well as consumption of beta-lactams and fluoroquinolones) were comparable among clusters ($P > 0.05$), this was not caused by increased use of antibiotics in general.

A. baumannii

Presence of aminoglycoside resistance genes and integrons

Similar to *P. aeruginosa*, the gene *aac(6')-Ib* coding for amikacin resistance was detected frequently, in 56.9% (91/160) of the isolates. Prevalence of the *aph(3')-VIa* was the highest (90.6%, 144/160) among tested genes. The gene *aph(3')-Ia* coding for kanamycin resistance and the gene *ant(3'')-Ia* coding for streptomycin resistance were also found, but these genes are less relevant clinically because the abovementioned antibiotics are rarely

or not at all used in Hungary. The *ant(2'')-Ia* gene could never be found in any isolates and out of the tested aminoglycoside methylase genes only *armA* could be detected (11.9%, 19/160). Class I integrons were found in the majority of isolates (93.7%, 150/160), class II and III integrons were not present.

Distribution of the carbapenem resistance genes among *A. baumannii* isolates

As expected, all isolates carried the weak chromosomal carbapenemase gene *bla_{OXA-51}* and the insertion sequence ISAbA-1; *bla_{OXA-23}* and *bla_{OXA-24}* was present in 78.1% (125/160) and 1.2% (2/160), respectively. *bla_{OXA-48}* and *bla_{OXA-58}* was not detected. Carbapenem susceptible isolates carried only the *bla_{OXA-51}* gene, carbapenem resistance was linked to carriage of *bla_{OXA-23}* or *bla_{OXA-24}*, besides *bla_{OXA-51}*.

Determination of the genotype

PFGE distinguished six clusters and four isolates with unique profiles.

Cluster A1 included 26 isolates from 19 patients. The majority of the isolates originated from the neurology ICU (15/26) and the pediatric ICU (7/26). The isolates of this cluster were carbapenem susceptible, but uniformly resistant to ciprofloxacin, amikacin and tobramycin. All isolates harboured the *bla_{OXA-51}* gene, the ISAbA-1 sequence and a class I integron with the gene cassette array *aac(6')-Ib*; hypothetical protein; *bla_{OXA-20}* (In426).

Cluster A2 contained seven isolates from four patients. Six of the seven isolates in this cluster originated from the surgery ICU, only one isolate originated from the neurosurgery ICU. They were multiresistant and susceptible only to polymyxins. Besides the *bla_{OXA-51}*, all of them carried *bla_{OXA-23-like}* gene and they were positive for *aph(3')-Ia* and *aph(3')-VIa* gene but none of the isolates harboured integrons.

Cluster B included 19 isolates from eleven patients. The majority of the isolates (14/19) in the cluster originated from the perinatal ICU. Antibiotic susceptibility was characterized by carbapenem and aminoglycoside resistance. Similarly to cluster “A2”, both *bla*_{OXA-51} and *bla*_{OXA-23-like} carbapenemases were detected in all members. Beside *aph*(3’)-*Ia*, *aph*(3’)-*VIa* genes another aminoglycoside resistance *ant*(3’)-*Ia* gene was found. They uniformly carried a class I integron with a gene cassette array of *aac*(3’)-*Ia*; hypothetical protein; hypothetical protein; *ant*(3’)-*Ia* (In561).

Cluster C1 contains twelve isolates of ten patients, all except one from the ICU of the 1st Internal Medicine Department. The isolates were susceptible only to polymyxins. The *bla*_{OXA-51-like} and *bla*_{OXA-23-like} genes were observed together in this cluster. All isolates were class I integron positive. Gene cassette arrays of eleven isolates were similar to the gene cassette array of cluster “B” (In561); one isolate carried an integron with a sole *ant*(3’)-*Ib* gene (In127).

Cluster C2 contained 29 isolates from 24 patients. The origin of the isolates was variable: surgery ICU (11); neurosurgery (6); neurology (6), 1st internal ICU (2); and other wards (4). Antibiotic susceptibility was highly diverse within the cluster; while all isolates from neurology were susceptible to carbapenems, isolates from other departments showed susceptibility only to polymyxins. Resistance gene pattern was similar to isolates of cluster C1 except the *bla*_{oxa-23-like} gene which was absent in the six carbapenem susceptible isolates from the neurology ward. The class I integron carried by all isolates was also identical to that found in Cluster 1.

Cluster D included 63 isolates, 50 of which originated from twelve patients of the pulmonology ICU. The majority of the isolates were resistant to all drugs except polymyxins. All isolates carried *bla*_{oxa-23-like}, *bla*_{oxa-51-like} and ISAba-1, *aac*(6’)-*Ib*, *aph*(3’)-*Ia*, *ant*(3’)-*Ia*, *aph*(3’)-*VIa*, and *aac*(3’)-*Ia* genes. Moreover, 30.2% (19/63) of the isolates

in the cluster carried a 16S rRNA methylase gene *armA*. All isolates carried a class I integron with a variable region containing *aac(6')-Ib*; *ant(3')-Ia* gene and a chloramphenicol resistance gene *catB8* (In439).

Four isolates did not belong to any cluster. One from the surgery ICU was similar to cluster A2 (showing a similarity of 86.6%), but carried an additional *aac(6')-Ib* gene. An identical pair of isolates from different departments (pulmonology ICU and 1st internal medicine ICU) showed extensive drug resistance (susceptible only to colistin). These two isolates carried *bla_{oxa-24-like}* carbapenemase besides the *bla_{oxa-51-like}* as well as ISAb_a-1, but none of the aminoglycoside resistance genes tested. They also were integron negative. The fourth isolate was also extensively resistant, was similar to cluster D regarding resistance genes, excepting that it was lacking the *aph(3')-Ia* and the *armA* genes. It also carried the same integron as found in cluster D.

Association of prevalence and resistance data with antibiotic consumption

A. baumannii was isolated from approximately 1% of all positive samples submitted during the years 2000 through 2008, but in 2009 and 2010 a sudden increase in the isolation rates from all positive samples (to 2.3% and 2.5%, respectively) was detected. Similar increase was found in positive blood samples (from 1-2% to 4.4%). In parallel, proportion of carbapenem resistant isolates increased from 6.2% to 63.8% from 2000 to 2010, which increased further to 73.0% in 2011.

In cross-correlation analysis, increases in carbapenem consumption followed the trend in 3rd generation cephalosporin ($r=0.63$, $p<0.001$ at the -9 quarterly lag corresponding to the period roughly two years earlier), and replaced piperacillin+tazobactam usage ($r=-0.54$, $p<0.001$ at +4 quarterly lag corresponding to the period a year later). Significant temporal correlation between carbapenem usage and consumption of polymyxins was found,

polymyxin use lagging behind carbapenem usage with five quarterly lags ($r=0.80$, $p<0.001$).

Carbapenem resistance of *A. baumannii* showed correlation with carbapenem usage with a delay of nine months ($r=0.43$, $p=0.005$ at the -3 quarterly lag), but not with usage of any other drug classes. Out of the different carbapenem drugs, meropenem showed temporal association with prevalence (eight to four lags; $r=0.45-0.49$, $p=0.002-0.006$) and carbapenem resistance (eight lags; $r=0.44$, $p=0.007$) of *A. baumannii* or with the number of positive patients per 100 bed-days (two lags; $r=0.39$, $p=0.01$). Ertapenem showed a similar effect, but with shorter delays. Prevalence lagged behind ertapenem usage with one to five quarterly lags ($r=0.54-0.58$, $p<0.001$), carbapenem resistance and the number of positive patients per 100 bed-days with six ($r=0.42$, $p=0.009$) and four ($r=0.56$, $p<0.001$) lags, respectively. These variables were not influenced by imipenem consumption. Differences in aminoglycoside usage were examined to clarify the background of the presence of the gene *armA*. It has been found that overall aminoglycoside usage was highest in the pulmonology department ($p=0.005-0.045$ in pairwise comparisons), where the isolates harbouring the aminoglycoside resistance methylase gene *armA* were the most frequent.

DISCUSSION

Pattern of isolations and continuous presence of *P. aeruginosa* and *A. baumannii* strains for months suggest an endemic occurrence. The isolates of the most common clusters (*P. areigonsa*: Cluster A-B; *A. baumannii*: Cluster A1-D) persisted for a long time but never caused outbreaks in the examined ICUs. Similar endemic epidemiology situation was reported by Foca et al. in 2000 in a neonatal intensive care unit, where the source was the healthcare personnel, and patients hospitalized for prolonged periods served as a putative reservoirs. In case of *A. baumannii*, most clusters were dominated by isolates from one or two wards, but many clusters was represented in multiple wards by a few isolates; suggesting that the ward-specific strains are continuously transferred to other wards, creating a polyclonal endemicity pattern. Exportation was detected in case of *P. aeruginosa*.

The association between antibiotic resistance and clonality suggests a role of aminoglycosides and carbapenems in the maintenance and spread of successful clones. Analysis of the molecular epidemiology revealed that aminoglycoside and carbapenem resistance cannot always be linked to a single clone. In case of *P. aeruginosa* it was rather due to acquisition of the acetyltransferase-encoding *aac(6')-Ib* gene, while in case of *A. baumannii* was due to acquisition of *bla*_{OXA-23-like} carbapenemase that were carried by all resistant clones. The resistant *A. baumannii* isolates in cluster C2 differed from susceptible isolates in the cluster by the carriage of the *bla*_{OXA-23-like} carbapenemase gene. These susceptible isolates originated from and were exclusively found in samples from the Neurology Department, where carbapenem consumption was significantly lower than in other wards. Similar situation occurred regarding aminoglycoside resistance methylase carrier isolates. The majority of patients harbouring isolates that carried the aminoglycoside resistance methylase gene *armA* were patients of the Pulmonology

Department with the highest aminoglycoside consumption. The most common aminoglycoside agent used in case of *P. aeruginosa* was amikacin, all isolates of cluster A harboured the amikacin resistance gene *aac(6')-Ib*. The group of patients infected with these isolates received significantly more amikacin than those patients from whom this clone was not isolated. These results suggest that selection pressure caused by antibiotics consumption may directly cause the spread of resistance genes by horizontal gene transfer. Integron carriage is strongly associated with aminoglycoside and carbapenem resistance. Accordingly, the vast majority of *P. aeruginosa* and *A. baumannii* isolates carried class I integrons. Integron carriage is widespread among Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella spp*, *Proteus spp* and the most common class is integron I. The integrons carried usually harbour beta-lactam and aminoglycoside resistance genes in their variable region, which may play a role in the persistence of successful strains. In case of class I integrons, the gene conferring resistance to cationic detergent disinfectants located at 3' conserved region of the integron may also have contributed to the persistence of the strains.

Several studies in the literature reported that beside antibiotic resistance, carriage of virulence factors can also play an important role in the persistence of the successful clones. Therefore, in this study the relationship between virulence genes carriage and the persistence of successful clones among *P. aeruginosa* isolates was also investigated. (Virulence of *A. baumannii* is barely known so far; no virulence factors have been proven to play a role in virulence of nosocomial strains.) Distribution of the virulence genes raises interesting questions regarding infections caused by *P. aeruginosa*. Based on our results, patterns of the virulence genes showed similar distribution between successful and sporadic strains. Both frequently isolated strains carried the gene *exoS* coding for a type III secretion system effector shown to be important for VAP pathogenesis, but not *exoU*,

which is considered the most harmful effector. Present data suggest that virulence patterns are not in direct association with success as a colonizer or pathogen. Antibiotic resistance in certain situations may be more important than virulence in spread of successful clones. As the choice of antibiotic is often motivated by the concern about drug resistance, this leads to increased usage of broad-spectrum antibiotics, which, in turn, provokes another outbreak, creating the resistance spiral. Present study reports such a situation. Based on overall antibiotic consumption data of the University of Debrecen, the antibiotic consumption from 2006 showed an increasing trend. This was attributable mainly to the increased consumption of 3rd cephalosporins against ESBL producers, which led to increase in carbapenem usage, bringing about the increased prevalence and carbapenem resistance in *A. baumannii*. Prevalence of *A. baumannii* and carbapenem resistance has been increasing in parallel with increased carbapenem usage. This result corresponds well to the observation that use of 3rd generation cephalosporins and carbapenems led to increase carbapenem resistance in *A. baumannii*.

The usage of different carbapenems showed different effects; while imipenem consumption seems to be less important as a cause for carbapenem resistance, meropenem and especially ertapenem consumption was associated with increased prevalence of *A. baumannii*. This corresponds well to the observation that imipenem is slightly more effective against *A. baumannii* than meropenem.

In the recent years, consumption of aminoglycosides as alternative agents has showed an increase similar to that of carbapenems. The aminoglycoside consumption was the highest at Pulmonology Clinic, where amikacin was the most frequently used aminoglycoside (50% of the total aminoglycoside consumption). Aminoglycoside resistance in *P. aeruginosa* (*aac(6')-Ib*) and *A. baumannii* (*armA*) has increased in parallel with aminoglycoside consumption.

Increases in the use of antibiotics are followed fast by increases in the resistance rates, but it is a long process to reduce the resistance. The spread of resistance is influenced differently by different drugs in the same drug family (ertapenem versus imipenem, amikacin versus gentamicin). A possible solution for reducing the resistance to aminoglycosides may be the diversification of the use of aminoglycosides; reducing the amikacin usage and increasing gentamicin and tobramycin usage. Optimization of antibiotic use should be given more attention in solving the difficult epidemiological situations.

In conclusion, the antibiotic consumption may be the main driving force for maintenance of endemic strains during non-outbreak periods. Antibiotic resistance in certain situations may be more important than virulence in spread of successful clones.

SUMMARY

Molecular epidemiological characterization of *P. aeruginosa* isolates and *A. baumannii* isolates originated from the University of Debrecen and the relationship between antibiotic consumption and antibiotic resistance of the clones have been investigated.

Three clusters (*P. aeruginosa*) and six clusters (*A. baumannii*) have been distinguished by pulsed field electrophoresis. Isolates belonging to the major clusters have persisted for long periods, however, they did not cause an outbreak at the examined ICUs. Neither carbapenem nor aminoglycoside resistance could be linked to a single clone only. Carbapenem resistance was attributable to the carriage of the *bla*_{OXA23-like} gene in case of *A. baumannii* isolates, while amikacin resistance was due to the carriage of the *aac*(6')-Ib gene in case of *P. aeruginosa*. Aminoglycoside resistance was strongly associated with integrons; the variable regions harboured genes conferring resistance to beta-lactams and aminoglycosides.

Ertapenem and meropenem but not imipenem consumption was associated with increased prevalence of *A. baumannii* as a cause carbapenem resistance. At the Neurology ICU, where the carbapenem consumption was significantly lower and ertapenem was a less preferred agent, majority of the *A. baumannii* isolates were susceptible to carbapenems. Prevalence of aminoglycoside resistant *P. aeruginosa* was strongly correlated to amikacin consumption which was frequently used at the Pulmonology ICU.

According to our results, relationship between antibiotic resistance and clonality is associated with consumption of aminoglycosides and carbapenems, which may also be the the main driving force for maintenance and spread of successful clones.



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Candidate: Julianna Mózes
Neptun ID: SGYJFL
Doctoral School: Doctoral School of Pharmaceutical Sciences

List of publications related to the dissertation

1. **Mózes, J.**, Ebrahimi, F., Gorács, O., Miszti, C., Kardos, G.: Effect of carbapenem consumption patterns on the molecular epidemiology and carbapenem resistance of *Acinetobacter baumannii*.
J. Med. Microbiol. 63, 1654-1662, 2014.
DOI: <http://dx.doi.org/10.1099/jmm.0.082818-0>
IF:2.266 (2013)
2. **Mózes, J.**, Szűcs, I., Molnár, D., Jakab, P., Fatemeh, E., Szilasi, M., Majoros, L., Orosi, P., Kardos, G.: A potential role of aminoglycoside resistance in endemic occurrence of *Pseudomonas aeruginosa* strains in lower airways of mechanically ventilated patients.
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3. Földi, R., Kovács, R., Gesztelyi, R., Kardos, G., Berényi, R., Juhász, B., Szilágyi, J., **Mózes, J.**, Majoros, L.: Comparison of In Vitro and Vivo Efficacy of Caspofungin Against *Candida parapsilosis*, *C. orthopsilosis*, *C. metapsilosis* and *C. albicans*.
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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LIST OF ORAL PRESENTATIONS RELATED TO THE DISSERTATION

Mózes J, Gorács O, Ebrahimi F, Miszti C, Kardos G. A karbapenem felhasználás és az *Acinetobacter baumannii* klónok előfordulása közötti összefüggés vizsgálata a Debreceni Egyetemen. A Magyar Mikrobiológiai Társaság 2014. évi Nagygyűlése, Keszthely 2014.

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