SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

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Table of Contents

1. Name and Surname

Wioletta Adamus-Białek

2. Possessed diplomas, scientific degrees

2003 **M.Sc. in biology, Institute of Biology, Faculty of Mathematics and Natural Sciences, Świętokrzyska Academy in Kielce,**
The title of the final work: 'Analysis of transposon mutants of *Proteus mirabilis* S1959 in reactions with anti-O3 and anti-R110 sera'.

2003 Postgraduate Certificate 'Nature in the Reformed School', Faculty of Mathematics and Natural Sciences, Świętokrzyska Academy in Kielce

2008 **Ph.D. in biological sciences, in the field of microbiology, Faculty of Biology and Environmental Protection, University of Lodz, Łódź**

The title of the doctoral dissertation:
'Development of a new genetic method of differentiation of uropathogenic *Escherichia coli* strains - TRS-PCR based on TRS genomic trinucleotide repeated sequences'
promoter: dr hab. Paweł Parniewski
reviewers: prof. dr hab. Antoni Różalski
prof. dr hab. Jarosław Dziadek

2009 Postgraduate Certificate 'Commercialization and Transfer of Knowledge Manager', Faculty of Management and Administration, Jan Kochanowski University of Humanities and Natural Sciences in Kielce

2016 Postgraduate Certificate 'Management of Healthcare Organizations', Warsaw School of Economics
The title of the final work: 'Introduction of a new medical product to the market in the context of the commercialisation of the scientific research'

3. Information concerning to-date employment in research institutions

01.10.2008 – 30.09.2009	**Assistant, Independent Department of Environmental Protection and Modelling, The Faculty of Mathematics and Natural Sciences, The Jan Kochanowski University in Kielce**
01.10.2009 – 30.09.2016	**Professor assistant**, Department of Environmental Protection and Modelling, The Faculty of Mathematics and Natural Sciences, The Jan Kochanowski University in Kielce
01.10.2016 – 31.09.2017	**Professor assistant**, Department of Microbiology, Institute of Medical Sciences, The Faculty of Medicine and Health Sciences, The Jan Kochanowski University in Kielce
01.10.2017 – until now	**Professor assistant**, The Head of Laboratory of Genetics, The Department of Surgery and Surgical Nursing (Head of the department: prof. dr hab. n. med. Stanisław Głuszek), Institute of Medical Sciences, The Faculty of Medicine and Health Sciences The Jan Kochanowski University in Kielce

4. Indication of scientific achievement resulting from Article 16, Clause 2 of the Act of 14 March, 2003 on academic degrees and titles, and on academic degrees and titles in arts (Journal of Act 2018, position. 1789):

The scientific achievement is a series of thematically related original publications, published in peer-reviewed journals in the Journal Citation Reports (JCR) database on molecular epidemiological analysis of the uropathogenic strains of *Escherichia coli* and analysis of their pathogenicity. **In these publications I am the first and corresponding author*.**

The total bibliometric value of the series of 6 publications making up the scientific achievement is Impact Factor – 13,202; Ministry of Science and Higher Education – 120

4.1. Title of scientific achievement:

Molecular epidemiological studies of uropathogenic Escherichia coli strains and the analysis of their pathogenicity

4.2. List of publications constituting the basis of the scientific achievement

1. Adamus-Bialek W*, Zajac E, Parniewski P, Kaca W, Comparison of antibiotic resistance patterns in collections of Escherichia coli and Proteus mirabilis uropathogenic strains, Mol Biol Rep, 2013, 40(4):3429-35

IF: 1,958; MNiSzW 20

My contribution to this publication concerned the research hypothesis formulation and the elaboration of the concept of work, research planning, optimisation and the execution of experiments (the analysis of the drug resistance of bacterial strains), the description of the procedures in the materials and methods section, the analysis and interpretation of the research results, the preparation of the graphic part of the presented results (Table 1, Table 2, Table 3), the preparation of the manuscript for the publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 70%.

2. Adamus-Bialek W*, Lechowicz Ł., Kubiak-Szeligowska AB, Wawszczak M, Kamińska E, Chrapek M, A new look at the drug-resistance investigation of uropathogenic E. coli strains, Mol Biol Rep, 2017, 44(1):191-202

IF: 1,889, MNiSzW 15

My contribution to this publication concerned the research hypothesis formulation and the elaboration of the concept of work, research planning, optimization and the execution of experiments: the analysis

of the bacterial drug resistance, the description of the procedures in the materials and methods section, the analysis and interpretation of the research results, the preparation of the manuscript for the publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 75%.

3. Adamus-Białek W*, Baraniak A, Wawszczak M, Głuszek S, Gad B, Wróbel K, Bator P, Majchrzak M, Parniewski P, The genetic background of antibiotic resistance among clinical uropathogenic Escherichia coli strains, Mol Biol Rep, 2018, pp 1-11

IF: 1,889, MNiSzW 15

My contribution to this publication concerned the research hypothesis formulation and the concept of work, research planning, participation in the experiments (the drug resistance analysis using the disk diffusion method, the identification of the ESBL strains by the DDST method and on chromID® plates ESBL plates (bioMérieux), the genetic analysis of the drug resistance by PCR, the nucleotide sequence analysis using BLAST (NCBI)), the description of the procedures in the materials and methods section, the analysis and development of the graphic part of the presented research results, the analysis and interpretation of the obtained results, the preparation of the manuscript for publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 75%.

4. Adamus-Białek W*, Kubiak A, Czerwonka G, Analysis of uropathogenic Escherichia coli biofilm formation under different growth conditions, Acta Biochim Pol, 2015, 62(4): 765-71

IF: 1,187, MNiSzW 15

My contribution to this publication concerned the research of the hypothesis formulation and the elaboration of the concept of work, research planning, optimization and the participation in the implementation of the experiments (the biofilm staining by the crystal violet method, gene detection by PCR, the bacterial strains culture under various growth conditions), the description of the procedures in the materials and methods section, performing the statistical analysis and developing a graphic part of the presented research results, the analysis and the interpretation of the research results, the preparation of the manuscript for the publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 80%.

5. Adamus-Białek W*, Vollmerhausen TL, Janik K, Hydrogen peroxide stimulates uropathogenic Escherichia coli strains to cellulose production, Microb Pathog, 2019, 126:287-291

IF: 2,332, MNiSzW 20

My contribution to this work concerned the participation in the research hypothesis formulation, performing the experiments (the biofilm staining by the crystal violet method, analysis of the cellulose gene expression by the calcofluor binding assay and RT-PCR, the bacterial strains incubation under

various concentrations of hydrogen peroxide). The description of the procedures in the materials and methods, the analysis and interpretation of the research results, the statistical analysis and development of the graphic part of the presented research results, the preparation of the manuscript for publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 60%.

6. Adamus-Białek W*, Wawszczak M, Arabski M, Majchrzak M, Gulba M, Jarych D, Parniewski P, Głuszek S, Ciprofloxacin, amoxicillin, and aminoglycosides stimulate genetic and phenotypic changes in uropathogenic Escherichia coli strains, Virulence, 2019, 10(1): 260-276

IF 3,947, MNiSzW 35

My contribution to this work concerned the research of the hypothesis formulation and the elaboration of the concept of work, research planning, the participation in the implementation of the experiments (resistance induction method and analysis of mutants), the description of the procedures in the materials and methods section, performing the statistical analysis and developing a graphic part of the presented research results, the analysis and the interpretation of the research results, the preparation of the manuscript for the publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 65%.

4.3. Description of the scientific purpose of the publications mentioned above and the results achieved with a discussion of their potential aplication

4.3.1. INTRODUCTION AND SCIENTIFIC PURPOSE

My interest in genetics and pathogenicity of bacteria begun during my Master's studies. I tried to explain the effect of transposon mutagenesis on the swarming growth and the lipopolysaccharide structure of *Proteus mirabilis*. During Ph.D. studies I started the research on *Escherichia coli* strains isolated from the urine of patients diagnosed with urinary tract infection (UTI). *Escherichia coli* is the predominant (80%) aetiological factor of urinary tract infections, thus usually each such strain was treated as uropathogenic. However, some studies have revealed that uropathogenic strains of *Escherichia coli* (UPEC) are a group of evolutionarily specialised clinical strains (Dobrindt, 2005, Elena et al. 2005). Other pathotypes of this species with specific properties predisposing to various, mostly intestinal diseases were also described in the literature. Beside typical intestinal commensals, many specific *E. coli* serotypes were observed to cause dangerous diarrhoea (e.g. serotype O157: H7 or O104: H4). It has also been shown that certain *E. coli* strains can cause meningitis especially in newborns. Although this species has been the main bacterial research model for a long time, no clear criteria for

differentiation of this species to particular pathogenic types have yet been developed. This is probably due to the extremely mobile genome of the E. coli, which can be modelled based on the environmental conditions which the bacterial population persists. The horizontal gene transfer, CRISPR/cas mechanism, pathogenicity islands, resistance plasmids or gene expression regulation are typical examples of factors affecting their adaptive capacities (Subashchandrabose and Mobley, 2015; Sokurenko, 2016; García-Martínez et al., 2018). Particular E. coli pathotypes were observed to possess O-specific LPS chains and pathogenicity factors characteristic for their group, but their absence does not exclude the ability to infect (Miyazaki et al., 2002; Kurazono et al., 2003). The urinary tract infection can potentially be caused by any *E. coli* strain, depending on the favourable environmental conditions, the host's predisposition as well as the adaptability of the bacteria.

The phylogenetic analyses in the literature prove that UPEC exhibit the greatest genetic diversity in relation to other *E. coli* strains. Studies show that this pathotype has evolved much earlier than the intestinal pathotypes (Elena et al., 2005, Brzuszkiewicz et al., 2006). Comparative analyses of the genomes of *E. coli* strains CFT073, *E. coli* O157: H7 and *E. coli* K-12 showed a specific DNA regions of UPEC strain. The phylogenetic distinction and the presence of unique sequences in the uropathogenic strains of *E. coli* can indicate an independent taxonomic affiliation of these pathogens (Dobrindt, 2005). However, the distinguish of a typical uropathogenic strain of *E. coli* from other *E. coli* strains was not practiced by clinicians, although very important in the further therapeutic process. Many known bacterial genotyping techniques, such as ERIC-PCR, REP-PCR, BOX-PCR did not differentiate the strains in terms of their pathogenicity. The development of a new TRS-PCR genotyping technique for the uropathogenic strains of *Escherichia coli* during my Ph.D. studies gave a starting point for further research on the pathogenicity of UPEC. It was observed that the tested collection of *Escherichia coli* strains isolated from the urine was divided into two different groups. The more precise analysis revealed a correlation between the specific profile of the CGG-PCR products, the presence of virulence genes and resistance to antibiotics from the quinolone group (Adamus-Białek et al., 2009). Strains resistant to fluoroquinolones rarely showed the presence of virulence genes, inversely to strains sensitive to fluoroquinolones - they were more virulent. Other authors have observed that during the induction of resistance to ciprofloxacin among *E. coli* strains – they lost some virulence factors (Soto et al., 2006). It was postulated that this mechanism is related to the DNA repair system, a SOS type response. It can be assumed that mechanism of virulence gene lose can be also related to the instability of the TRS sequence. The obtained research results during doctoral studies were an impulse for further research on uropathogenic strains of *E. coli*. The studies on drug resistance and pathogenicity were an important part of my molecular epidemiological studies on uropathogenic *E. coli* strains.

Considering the above, the aim of the research, which was included in the scientific achievement, was the molecular epidemiological analysis of the uropathogenic *E. coli* strains in terms of virulence and drug resistance. This goal was achieved through:

- The detailed analysis of drug resistance
- The differentiation of *E. coli* strains in comparison to *P. mirabilis* strains based on sensitivity to selected antibiotics
- Searching for associations between antibiotics based on the sensitivity level of the strains
- The use of chemical and statistical methods in the analysis of susceptibility and prediction of drug resistance
- The analysis of genetic determinants of drug resistance
- Studying the relationship between the presence of selected virulence factors and the ability to biofilm formation
- Defining the *E. coli* ability to survive and the biofilm formation under unfavourable environmental conditions (urine, free oxygen radicals, subliminal concentration of antibiotics)
- The study of the influence of free radicals on cellulose production in the studied strains
- The study of the influence of antibiotics on the phenotypic and genetic variability of the tested strains.

4.3.2. DESCRIPTION OF SCIENTIFIC ACHIEVEMENT

The main subject of the research was the collection of *Escherichia coli* strains isolated from the urine of patients with urinary tract infection. *Escherichia coli* is the most important aetiological factor of UTI. This is generally due to the close proximity of the large intestine where *E. coli* represents the intestinal microflora. The presence of Type 1 fimbriae on the surface of *E.coli* cells, which plays the most important role in adhesion (Terlizzi et al. 2017), is also significant. The presence of *E. coli* in the urinary tract may be incidental due to e.g. weakness of the body and the immunity deficiency. The infection may also be promoted by anatomical defects of the urinary tract or diseases conducive to this type of infection, such as diabetes (Williams and Schaeffer, 2004). *Escherichia coli* is a widespread species in the environment and human life. In the process of evolution, their genome underwent changes, the pressure of the environment forced numerous mutations. On the path of horizontal gene transfer *E. coli* acquired from other species nucleotide sequences giving them pathogenicity features. Thanks to these changes, they specialised into the pathogenic strains (Dobrindt et al., 2005, Elena et al., 2005). Nowadays, their existence in the environment is not only an indicator of the risk of occurrence of such pathogens as *Salmonella* spp. Or *Shigella* spp.. The presence of pathogenic *Escherichia coli* strains in the environment is an equally serious threat to public health, such as uropathogenic strains of *Escherichia coli* mentioned above (Terlizzi et al., 2017). It can be concluded that the ability to survive in unfavourable environmental conditions is the priority of this group of pathogens. Environment poor in nutrients, mechanical forces of the urine stream, the presence of antibiotics and cells of the immune system stimulate the development of many adaptive mechanisms. Their peculiar adaptation is the ability

to develop an intracellular bacterial communities (IBC) inside the umbellate cells of the bladder, which protects them against the immune response of the host (Anderson et al., 2004, Hunstad and Justice, 2010). The ability to lose pathogenicity factors during the drug resistance induction was also observed, which reduces their immunogenic properties even further (Zhanel et al., 1992; Soto et al., 2006; Wiles et al., 2008; Hunstad and Justice et al., 2010; Goneau et al., 2015). The problem of spreading and increasing antibiotic resistance is one of WHO's priorities. The studies on the pathogenicity mechanisms of the uropathogenic strains of *Escherichia coli* are fully in line with this problem. **Drug resistance seems to be a key element of the UPEC pathogenicity and was the basic matter of the research included in the scientific achievement. The obtained results of microbiological, molecular and chemical research was subjected to detailed analyses using advanced mathematical tools. This allowed to make many important conclusions about the pathogenicity of *Escherichia coli* and to indicate the applicability of mathematical tools in epidemiological studies.**

The research results presented in the first publication specified in the scientific achievement (**Adamus-Bialek W, Zajac E, Parniewski P, Kaca W, Comparison of antibiotic resistance patterns in collections of Escherichia coli and Proteus mirabilis uropathogenic strains, 2013, Mol Biol Rep, 40(4):3429-35**) allowed to observe the significant differences and dependencies in the antibiotic resistance of the *Escherichia coli* and *Proteus mirabilis* strains. The 129 *E. coli* strains and 3 different collections of *P. mirabilis* strains were analysed based on the susceptibility to antibiotics from the groups of penicillins, cephalosporins, carbapenems, monobactams, aminoglycosides, fluoroquinolones, polypeptides and to tetracycline, nitrofurantoin, co-trimoxazole and fosfomycin. These bacteria are closely related phylogenetically, exist in the same environments and may cause similar infections, including the urinary tract. Antibiotics used in the treatment of this type of infection induce the expression of similar drug resistance mechanisms. Nevertheless, *E. coli* strains showed about 3 times more different drug resistance profiles and 4 times more unique drug resistance profiles compared to *P. mirabilis* strains. In addition, *P. mirabilis* strains also showed a more homogeneous response to the effects of individual antibiotics – most strains (an average of 80%) showed sensitivity or resistance to given antibiotic, while *E. coli* strains exhibited different sensitivity to antibiotics. The differentiation of *E. coli* strains based on the level of the antibiotic susceptibility confirmed their heterogeneous nature as opposed to *P. mirabilis* strains. It can be assumed that the directions of drug resistance development will be less predictable, resulting from the use of various antibiotic resistance mechanisms by *E. coli* strains. This prediction of the directions of resistance development can be obtained by the creation of Kohonen map by applying the association analysis. This map clearly indicates the relationship between different antibiotics. It can be deduced that the population of *E. coli* strains may cause more different directions of drug resistance as compared to *P. mirabilis*. The observed diversity of *Escherichia coli* strains may be due to the fact that among bacteria isolated from urine of patients diagnosed with UTI find both uropathogenic and non-neuropathogenic strains. It should also be emphasised that the use of Kohonen

modelling seems to be a promising technique in predicting the increase of bacterial drug resistance and could be routinely used for epidemiological analysis of drug resistance among E. coli strains as well as many other pathogenic species, among which rapid drug resistance emergence is observed.

The obtained research results inspired to look for further associations within the drug resistance mechanisms. In the next publication indicated in the scientific achievement (**Adamus-Bialek W, Lechowicz Ł., Kubiak-Szeligowska AB, Wawszczak M, Kamińska E, Chrapek M, A new look at the drug resistance of uropathogenic E. coli strains; 2017, Mol Biol Rep, 44(1):191-202**) the same collection of *Escherichia coli* strains were analysed for sensitivity to 37 antibiotics of all classes. An innovative technique of the attenuated infrared spectroscopy with Fourier transform ATR FT-IR was used to analyse the spectra of individual bacterial strains. The interpretation of bacterial spectrum in the correlation with sensitivity to particular antibiotics was obtained by the development of the so-called artificial neural networks. Thanks to this, it was possible to select multi-drug resistant (MDR) strains from the *E. coli* collection tested within 24 hours. The obligatory standards of bacterial resistance analysis require the preparation of fresh inoculum, strain isolation, the antibiogram and its interpretation, which requires much more work, time and expenses. This innovative application of the ATR FT-IR combined with the statistical analysis can be an alternative tool for rapid monitoring of MDR strains. It only requires a simple spectrophotometric analysis of overnight culture of strains on the agar. The weak point seems to be an elaborate interpretation of the obtained results, which makes it difficult to use it in the routine diagnostics. However, it can be a good cause to start an effective cooperation of scientific institutions with epidemiological or diagnostic stations. Additional, advanced techniques of further statistical analysis allowed to indicate specific dependencies between antibiotics and strain sensitivity. The diameters zones (mm) of inhibited bacterial growth around the antibiotic discs were analysed by Ward agglomeration. It turned out to be an effective technique of *E. coli* epidemiological differentiation. Four clusters of strains showed different drug resistance profiles, e.g. in Cluster 2 all strains were resistant to tigecycline, meciline, ampicillin with sulbactam, meropenem, cefalexin and cefadroxil. In addition, this cluster showed a much more frequent occurrence of pathogenicity factors compared to Clusters 1 and 3. The presented method of drug resistance analysis may be an additional tool in epidemiological investigations of bacterial strains. Currently, bacterial differentiation uses advanced genotyping techniques such as MLST, PFGE (Camelena et al., 2019). Although an example of the presented own research using mathematical tools for strains differentiation based on drug resistance profiles does not require additional time-consuming and expensive laboratory techniques. It may be another alternative for clonal differentiation of bacterial strains. Subsequent analyses of own research were also carried out on the basis of the diameters zone of inhibited growth of all *E. coli* strains in relation to individual antibiotics. The values of the diameters zone were compared to the same values of the *E. coli* reference strains published by EUCAST. Reference strains originate from various European collections and their susceptibility is a benchmark for standardization of MBC and MIC values

of antibiotics and defining bacterial resistance and sensitivity to a given antibiotic. This allows global monitoring of antimicrobial agents (van der Bij et al., 2012; Matuschek et al., 2014) and to compare the obtained research results with the generally accepted standards published by EUCAST or CLSI. In the presented results of own research it was observed that the strains react differently to particular antibiotics. The behavioural groups were proposed in our research: 'Sensitive', 'Coming intermediate', 'Intermediate', 'Resistant' and 'Diverse'. The studied *E. coli* collection was classified into the behavioural groups based on the largest number of strains with a homogeneous response to the antibiotic. Studied group of bacteria did not represent 'Coming resistant' to any antibiotic. Behavioural groups were proposed in order to show the distance of the tested bacterial collection to the resistance limit to a particular antibiotic and to pay attention to the danger of the emergence of the resistance to a given antibiotic in the population. The clue in the use of individual antibiotics is therefore the classification of a representative population of bacteria in a given behavioural group. For instance, in the case of the studied collection of E. coli strains isolated from the urine, further routine use of cefoxitin, gentamicin, netilmycin, ticarcicline with clavulanic acid will probably lead to resistance to these antibiotics because they were classified as 'Intermediate' to these antibiotics. It can also be concluded that being a part of the 'Diverse' group will result in unpredictable tendency of increasing resistance to antibiotics from this group. In this case it will be amoxicillin, ampicillin, piperacillin, ticarcyline, chloramphenicol and fluoroquinolones. It is worth to consider to cease to use the antibiotics until the full sensitivity of this bacterial population returns. The comparison of the studied Polish strains collection to the group of European reference strains (EUCAST) also allowed to observe much greater resistance to antibiotics such as: amikacin, ceftazidime, imipenem, cefoxitine, netilmycin, tobramycin, tigecycline, amoxicillin, chloramphenicol, moxifloxacin and norfloxacin. The differences between these strain groups may derive from different standards and local patterns of antibiotic usage in a particular region. Similar comparative analyses were presented by other authors, e.g. Konca et al. (2016) presented different profiles of drug resistance depending on the region, time and other features that may indicate specific trends of antibiotic resistance of clinical bacteria. The presented results are an example of how another mathematical tool can be used in the clinical microbiology, in this case helpful in rational antibiotic therapy and counteracting the spread of the bacterial resistance. The increase of bacterial drug resistance is a serious problem of current medicine, and its source is not only Asian countries but also a large part of Europe, where the use of antibiotics is routine and empirical, often inadequate to infection. A serious problem is also the use of broad-spectrum antibiotics, in the wrong dose with too long treatment. Having continued the statistical analysis, Kapp Cohen's coefficient was used to indicate the specific antibiotic relationships according to their effect on the tested *E. coli* strains. Cohen's analysis allowed to indicate how the entire set of strains reacts to a particular antibiotic. The relationships between antibiotics: synergistic – having the same effect on the bacterial collection tested and antagonistic – showing opposite action were shown. The presented dependencies pertained only one group of 127 strains of bacteria isolated from urine in Łódź. It would be interesting to apply such analysis to a larger population of strains isolated from the

urine of patients from different regions. It would create a representative database that could be a quick and reliable indication for doctors in rational antibiotic therapy. For example, when analysing a tested collection of *E. coli* strains, it does not matter which antibiotic from the synergistic group will be used during the treatment because it will show exactly the same effect on the bacteria. On the other hand, in the case of resistance to a given antibiotic, one can successfully make an empirical decision to use an antibiotic from the antagonistic group. Similar analyses were presented by Obolski et al. (2016) using a different mathematical model. They observed several different co-occurring patterns of drug resistance and similar dependence on penicillins within *E. coli* strains. The use of mathematical analyses in clinical microbiology is not typical, whereas the presented results indicate the huge potential of these tools useful in differentiation, epidemiology and antibiotic therapy.

Continuing the research on *E. coli* drug resistance, the next step in my research was to analyse the mechanisms of resistance to antibiotics. The use of antibiotics triggers various mechanisms of drug resistance in bacteria. They can be: induction of proteins production that disintegrate or inactivate antibiotics (beta-lactamases), modification of PBP proteins, active drug removal from the cell, horizontal gene transfer (sulfonamide resistance plasmids) or mutations in genes (*gyrA*, *parC*) (Sanchez-Cespedes et al., 2015, Peterson and Kaur, 2018). It was interesting during the own research as in the case of the tested *Escherichia coli* strains – the known genetic markers would correlate with the phenotypic resistance to antibiotics. The correlation between the presence of bla_{TEM}, bla_{CMY}, bla_{SHV}, $bla_{OXA\text{-}1}$ and $bla_{CTX\text{-}M\text{-}1}$ genes and resistance to beta-lactam antibiotics was analysed. In addition, the correlation between the presence of the *aac(3)-II* gene and resistance to aminoglycosides and the correlation between *sul1*, *sul2* and *sul3* genes and sulfonamide resistance were analysed as well. The occurrence of mutations in the genes *gyrA* and *parC* and *qnrA*, *qnrB*, *qnrS* in correlation with drug resistance to fluoroquinolones was also examined. The results of the research are presented in the publication **Adamus-Białek W, Baraniak A, Wawszczak M, Głuszek S, Gad B, Wróbel K, Bator P, Majchrzak M, Parniewski P, The genetic background of antibiotic resistance among clinical uropathogenic Escherichia coli strains, 2018, Mol Biol Rep, pp 1-11**. Many factors influence the occurrence of the studied genes, such as the antibiotic policy in a given country, the source and year of isolation of individual bacterial strains, the ability to transfer genes, and mechanisms for acquiring drug resistance. The results of own research present the characteristics of a population of bacteria from 10 years ago, which allowed us to notice the changes currently observed in a similar population of bacteria. Currently, the major epidemic threat is ESBL (extended-spectrum beta-lactamases). Among the tested bacterial collection, we identified about 1.5% of strains producing β-lactamases with an extended spectrum of action and 1.5% of strains producing AmpC, which was the standard level in 2005 – 2007. The situation in Poland seems to have been relatively stable for 10 years. Currently, about 2% of UPEC strains are ESBL producers, but in many other countries the situation is much worse (Stefaniuk et al., 2016, Parajuli et al., 2017). Mechanisms of resistance to beta-lactam antibiotics seem to be the most

complex and diverse. This is also clearly visible in the case of genetic analysis of own research. The *bla*$_{TEM}$ gene was the most frequently detected gene in the tested group of *E. coli* bacteria (91% of strains, unpublished data), and strains without *bla*$_{TEM}$ were sensitive to the majority of beta-lactam antibiotics, mainly amoxicillin. Considering that the vast majority of isolates were ESBL-negative despite the presence of *bla*$_{TEM}$, it can be assumed that the identified gene encodes a broad-spectrum enzyme, most likely TEM-1. TEM-1 is the main determinant of *E. coli* resistance to amino-penicillins and the most common β-lactamase encoded by plasmid genes. It is estimated that this enzyme is present in approximately 50% of all *E. coli* clinical isolates (Bailey et al., 2011, Ojdan et al., 2014). The next most common gene was *bla*$_{CMY-2}$ (19.5% of studied strains), but only one of them was a producer of AmpC beta-lactamase, although it occurs on small plasmids coding for AmpC enzymes (Bauernfeind et al., 1996). The remaining strains of *bla*$_{CMY-2}$ were mostly resistant to at least one beta-lactam antibiotic, but were not detected as ESBL producer strains. The prevalence of this gene was not correlated with the resistance to betalactam antibiotics and ESBL production. The same results were observed for further analysed genes. The *bla*$_{OXA-1}$ gene was detected only in one strain. That strain, despite the resistance to all analysed beta-lactam antibiotics (with the exception of amoxicillin with clavulanic acid and imipenem), was not classified as ESBL producer. Also, in the case of the *bla*$_{SHV}$ gene – it was identified only in one strain resistant to only one beta-lactam antibiotic (ceftazidime), which may suggest the presence of SHV-1 or SHV-11 betalactamase with a broad spectrum of activity (Shahi et al. 2013). Comparing the presented own results to the work of other authors - *bla*$_{CMY-2}$ was also often identified in the strains of *E. coli*, *Klebsiella* spp. and *Salmonella* spp. isolated from various sources in the United States, Greece and Algeria (Bauernfeind et al., 1996; Koeck i wsp., 1997; Fey et al., 2000, Winokur et al., 2000). In the case of *bla*$_{OXA-1}$, other authors also observed a similar prevalence (Alizade et al., 2015; Liao et al., 2017). The next analysed *bla*$_{CTX-M-1}$ gene was not detected in any *E. coli* strain tested, which is consistent with data published by other authors (Canton and Coque, 2006), although the first strain producing β-lactamase CTX-M-3 was identified in Poland in 1996 and was the dominant type of ESBL in the country (Gniadkowski et al., 1998b; Ojdan et al., 2014). The literature shows that CTX-M enzymes were identified at various sites in the second half of the 20th century, including Argentina, Israel and Paraguay (Canton and Coque, 2006). The *bla*$_{CTX-M-1}$ gene was identified in 1989 in Germany for the first time (Canton and Coque, 2006) and it was responsible for the expression of these beta-lactamases in Europe. The results of drug resistance in 2014 presented by Ojdan et al. (2014) showed that the *bla*$_{CTX-M-15}$ gene was identified in all 12 analysed *E. coli* strains isolated from patients in Bialystok and only two strains were positive for *bla*$_{TEM-1}$ genes and *bla*$_{SHV}$. Korzeniewska et al. (2013) published the results of antibiotic resistance among *E. coli* strains isolated from various environments (hospital and municipal sewage, river, air). They identified about 30% of ESBL-positive strains, in which *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-3}$, *bla*$_{CTX-M-5}$, *bla*$_{CTX-M-15}$ genes were the most common. There are many publications in the literature presenting the occurrence of specific genes in correlation with phenotypic drug resistance. It should also be noted that the occurrence of antibiotic resistance genes is usually

different in strains isolated from different samples (Winokur et al., 2001). Considering the resistance to other antibiotics, aminoglycosides and sulfonamides also play an important role in the treatment of UTI. The *aac(3)-II* gene is described as the most strongly correlated gene with aminoglycoside resistance, but this statement was not confirmed in our results. Both groups of resistant strains, resistant or susceptible, had or did not have *aac(3)-II*, therefore it should be stated that in the case of the studied collection of *E. coli* strains isolated from urine, this gene cannot be a marker of resistance to any aminoglycoside antibiotic. Similar results have been published by other authors (Soleimani et al., 2014, Haldorsen et al., 2014). We also analysed the occurrence of *sul* gene in correlation to sulfonamide resistance. The products of these genes change the activity of DHPS (dihydropterate synthase). This enzyme has an affinity for PABA and when it is encoded by *sul*, the strain remains resistant to sulfonamides. The trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) in the next stage of the same metabolic pathway (Caron et al., 2017). It was interesting whether there is a correlation between phenotypic resistance to trimethoprim and the occurrence of *sul* gene – our results did not confirm such a relationship. The literature also indicates the association of *sul* gene with resistance to cotrimoxazole. Our studies revealed the common occurrence of *sul1* and *sul2* genes even in the case of strains sensitive to trimethoprim or cotrimoxazole. The results presented by other authors show a similar distribution of these genes (Huovinen et al., 1995, Sköld, 2001). In addition, in the case of our results, the rare occurrence of *sul3* in the *E. coli* strains may indicate that the synthesis of modified DHPS due to the expression of the *sul3* gene has recently appeared in resistant strains of bacteria (Grape et al., 2003). This confirms of doubtful and negative results frequently obtained by PCR in this group of bacteria. The sulfonamides are used for treatment of UTI as a combination of sulfamethoxazole and trimethoprim. This was the dominant UTI therapy in 1995 – 1996. Currently, due to the increasing prevalence of resistance to trimethoprim-sulfamethoxazole among *E. coli* strains, this should not be the first choice for the treatment of *E. coli* infection (Kallen et al. 2006). Noteworthy is also the high incidence of *sul* gene, which may result in the rapid emergence of resistance to this substance. The most obvious results of our genetic analysis were obtained during the identification of point mutations in the *parC* gene (encoding topoisomerase IV) and *gyrA* (encoding the DNA gyrase). It is known that the Ser/80/Ile mutation in *parC*, as well as the Ser/83/Leu and Asp/87/Asn mutations in *gyrA* strongly correlate with the phenotypic resistance to quinolones (McDonald et al., 2001). The results of our research confirm these reports. **Moreover, we have shown for the first time that the accumulation of mutations in these genes decreases the sensitivity to fluoroquinolones in the case of the studied *E. coli* strains.** What is more, numerous silent mutations in 'hot spot' loci (specific mutation site) were characteristic for *parC*, and missense mutations were identified in other *gyrA* codons. **It can be concluded that the study of *gyrA* and *parC* gene sequences in bacteria may play a predictive role in phenotypic resistance to fluoroquinolones and makes it useful in epidemiological studies.** The importance of silent mutations in the process of adaptation and phenotypic changes in various organisms is described in the literature (Supek, 2016). Other silent mutations have

also been identified by other authors (Thong et al., 2016; Yang et al., 2017). Analysis of the appearance of mutations in the bacterial population during the process of acquiring resistance to fluoroquinolones seems to be interesting. This accumulation of mutations may be due to the low specificity of fluoroquinolones. On the other hand, fluoroquinolones can induce the SOS response that can induce DNA mutations in the bacterial genome, inter alia in the *gyrA* and *parC* gene regions (Baharoglu et al., 2011). Silent mutations can also induce a phenotypic effect by the regulation of transcription (Agashe et al., 2016; Hauber et al., 2016); alternatively, they can also alter the affinity of the fluoroquinolones to the binding site. An interesting observation obtained in the research work is that the hot spot of *parC* was more specific but less sensitive to fluoroquinolones (many silent mutations in the hot spot) compared to the hot spot of *gyrA* – many missense mutations in different loci. We have not identified other specific mutations in the *gyrA* and *parC* genes in correlation with phenotypic resistance but other mutations are known in the literature, for example in gyrA – Ser/83/stop, Asp/82/Asn, Gly/81/Asp, Asp/82/Gly, Ser/431/Pro (Cesaro et al., 2008). In addition, resistance to fluoroquinolones can also be caused by the presence of plasmids that produce the protein Qnr (QnrA, QnrB, QnrS) that protects the DNA binding site against quinolone. Qnr plasmids induce low resistance, but their presence enhances the resistance to fluoroquinolones (Tran et al., 2005). According to the above the presence of *qnr* genes in studied *E. coli* strains was analysed. However, none of the strains was qnr-positive. Rare occurrence of these genes has been confirmed in other publications (Jlili et al., 2014, De Silva et al., 2017). Mammeri et al. (2005) examined 297 *E. coli* strains resistant to nalidixic acid and they identified only 1 strain with the *qnr* gene. Some authors report different results, this probably depends on the local distribution of the Qnr plasmid. **In conclusion, genetic analysis is not sufficient to identify bacterial resistance to antibiotics except the resistance to fluoroquinolones. Genetic analyses may play a role in predicting resistance and may indicate a high or low risk of resistance. The obtained results for the first time indicated the accumulation of nonspecific mutations in the topoisomerase and gyrase genes, which were correlated with the decreased sensitivity to fluoroquinolones. The distribution of the analysed genes was very diverse and showed a high adaptive potential of *E. coli* bacteria to the toxic environment (antibiotic).**

Exploring the knowledge about the pathogenicity mechanisms of the *E. coli* strains led my research towards the bacterial ability to survive and create biofilms under the various unfavourable environmental conditions. The natural environment of UPEC strains is urine and umbrella cells, to which bacteria attach themselves in the first stages of adhesion (Hunstad and Justice, 2010). Determination of the survival ability of the tested collection of *E. coli* strains and the development of biofilms in the artificial urine environment was the next stage of my research. The results of the analyses were presented in the paper **Adamus-Białek W, Kubiak A, Czerwonka G, Analysis of uropathogenic Escherichia coli biofilm formation under different growth conditions, 2015, Acta Biochim Pol, 62(4):765-71.** The heterogeneity of the studied *Escherichia coli* strains was also revealed at the level of biofilm

formation. The bacterial incubation was carried out in artificial urine and under optimal conditions (LB broth) for 96 hours at 37°C. The level of growth was tested spectrophotometrically based on optical density (A_{600}). In the case of the culture under optimal conditions, it was observed that the strains reached their maximum and stable level of density after 24 hours, which means that the logarithmic phase of growth was stopped. The ability of biofilm formation was examined also spectrophotometrically (A_{531}) after the crystal violet staining of bacterial cells attached to the plate surface (polyurethane). This technique detects the bacterial biofilm at the first stage of cell adhesion to solid surfaces. **The studies showed that the level of biofilm also reached its maximum after 24 hours and, statistically, significantly decreased in the following hours of the experiment, which could be related to the 'life cycle' of the biofilm, the tendency to spread bacterial cells and deplete the nutrients.** This observation is not consistent with the belief that bacteria usually need more time to create a biofilm. It should be emphasised that the biofilm of uropathogenic *E. coli* strains is not typical in comparison to commonly described biofilm structures of the majority of bacterial species such as *S. aureus* or *P. aeruginosa* (Spiers et al., 2003, Zhang et al., 2015). The average level of biofilm was much lower compared to the typically described biofilms, but UPEC strains do not have to create a vast and complex agglomeration of cells on the surface, because the intracellular bacterial communities (IBCs) protect bacteria against host immune cells. In the first stage, the bacteria attach to the appropriate receptors of the bladder epithelial cells and quickly penetrate into their interior before the neutrophil influx (Hannan et al., 2012). The observed own results may reflect this bacterial behaviour. Adhesive factors play an important role here (Bergsten et al., 2005), which was also confirmed in our research – **strains with the presence of genes encoding P and/or S fimbriae were able to create a statistically significantly stronger biofilm than strains without these genes.** Additionally, we tried to find a direct relationship between the presence of other genes (*sdiA*, *rscA* and *rpoS*) and the bacterial ability biofilm formation (Ito et al., 2007; Ranjit and Young, 2013; Wei et al., 2001). These genes are described in the literature as crucial in biofilm formation, but they were present in all *E. coli* strains in our study. It can be concluded that not the presence of a gene but its expression triggers the process of biofilm formation, which is primarily dependent on environmental factors (Schembri et al., 2003). Analysing the further results of the presented studies, a significant decrease in the growth and biofilm formation in urine condition was observed in comparison to the optimal conditions. Nevertheless, the difference of optical density between urine and optimal conditions was higher than the difference of biofilm formation level. On this basis, the level of relative biofilm was determined independent on the optical density of the bacteria in the culture. **It was shown that bacteria produced a relatively higher level of biofilm in synthetic urine than in optimal conditions. It can be concluded that in an abiotic environment, bacterial cells were stronger involved in biofilm formation than in cells divisions.** Microscopic observation of a biofilm formed on glass and stained with fluorescent dyes was also carried out. The studied *E. coli* strains on glass formed typical structures of bacterial clusters with characteristic filamentous cells described in the literature, which form at the stage of bacterial release from the bladder

cells in order to colonise the next (Justice et al., 2004). Elongated cells arise due to the inhibition of the division of the cell wall, at the same time dividing the nucleoid, which makes them become 'invisible' to the cells of the host immune system. **It can be assumed that this feature of E. coli determines their affiliation to the uropathogenic pathotype, however, their ability to penetrate into the eukaryotic cells should be verified.** The comparison of the biofilm formation on the polyurethane plate and glass – no correlations were observed. The strains forming a strong biofilm on the glass did not reflect similar properties on polyurethane and vice versa. This discrepancy may be related to the various stages of biofilm observed using different techniques. Examination of the biofilm with crystal violet mainly allows to check the bacterial adhesive properties. In the presented studies, usually strains that formed microcolonies on the glass surface were characterised by different levels of adhesion to polyurethane. The mechanism of biofilm formation can also be linked to other properties of bacterial strains. The relationship between the formation of biofilms and the surface (polyurethane, glass) may be related to the hydrophobicity of the surface and outer membrane of a specific strain (Krasowska and Sigler, 2014, Liu et al., 2004). Probably the hydrophobic bacteria attach better to the hydrophobic polyurethane and vice versa. Our preliminary studies showed that the analysed *E. coli* strains were characterised by a diversified hydrophobicity of the cell surface, but it was difficult to correlate with the ability to form a biofilm (unpublished results). **In conclusion, the scientific results presented in this publication provide a lot of valuable information on the pathogenicity of the studied *E. coli* strains. The conclusions are also consistent with the observations obtained during the drug resistance analysis – the collection of *E. coli* strains seems to be a highly heterogeneous group of bacteria. One species of bacteria (*Escherichia coli*) isolated from the same environment (urine) can differently adhere to different surfaces and exhibit different ability to grow and form biofilms.**

The obtained results prompted me to further analyse the uropathogenicity of *E. coli*. I was interested not only in how the studied group of bacteria 'cope' with antibiotics or abiotic urine but also with other factors occurring during the inflammation. In the early stages of infection, UPEC strains stimulate the TLR4 receptor, which triggers the inflow of PMN leukocytes (Agace, 1996). In addition, the epithelial and immunological cells produce a panel of cytokines, chemokines and inflammatory molecules such as nitric oxide and reactive oxygen species during infection (Hunstad and Justice, 2010; Blomgran et al., 2004; Bian et al., 2000), which can be released from hydrogen peroxide. Halliwel et al. (2000) detected H_2O_2 concentrations above 0.1 mM in freshly excreted human urine of healthy individuals. Antibacterial hydrogen peroxide concentrations are predicted to be even higher in the urine of UT patients (Long et al., 1999; Halliwell et al., 2000). We hypothesised that the presence of reactive oxygen species is another factor that can stimulate *E. coli* bacteria to activate defence mechanisms. The aim of the study was to analyse the response of *E. coli* to the presence of H_2O_2 as a source of reactive oxygen species, and the obtained results were published in **Adamus-Białek W, Vollmerhausen TL, Janik K, Hydrogen peroxide stimulates uropathogenic Escherichia coli strains to cellulose**

production, 2018, Microb Pathog, 126:287-291. A collection of 25 clinical uropathogenic strains of *Escherichia coli*, including reference strain *E. coli* No. 71.8 were tested. The 0.625 mM of H_2O_2 was determined as the minimum sub-inhibitory concentration of hydrogen peroxide relative to the growth of the reference strain. All strains were cultured at this H_2O_2 concentration in LB broth at 37°C for 24 hours, with respect to the control conditions. Although the level of growth was significantly decreased, and the tolerance of hydrogen peroxide differed between the strains, the majority of *E. coli* strains (85%) were able to survive the overnight exposure to hydrogen peroxide. An experiment of short 15-minute exposure of the tested strains to higher concentrations of hydrogen peroxide (0.625 - 275 mM) was also carried out and, as it turned out, some strains survived even the highest concentration of hydrogen peroxide tested (275 mM) and more than half of the strains were able to survive in 137 mM of H_2O_2 during this experiment. **The observed *E. coli* resistance to high concentrations of hydrogen peroxide was in line with the results of studies by Schembri et al. (2003).** In addition, we have shown that *E. coli* was significantly more tolerant to the H_2O_2 than the T24 line of human bladder epithelial cells. The epithelial cells did not survive during overnight culture in medium supplemented with 0.625 mM of H_2O_2, while the lower concentration of 0.125 mM of H_2O_2 was not cytotoxic (XTT test, data not published). According to literature data, the applied H_2O_2 concentrations can be present in the urinary tract but they are not bactericidal against UPEC. It should be considered that it is not the only one component involved in inflammation during infection. In the further our studies, the same clinical UPEC strains were also analysed based on biofilm formation after overnight culture in LB broth with 0.625 mM hydrogen peroxide. The level of biofilm formation was significantly decreased (about 50% reduction of biofilm) but it was much less varied in comparison to the culture under optimal conditions. Similarly, as in our previous studies – relative biofilm formation – **no inhibitory effect of H_2O_2 on the biofilm formation was observed. This is consistent with the previously described results (Adamus-Białek et al., 2015), probably hydrogen peroxide (like abiotic urine) limited cell divisions, resulting in a reduced number of attached bacterial cells to the polyurethane surface**. It can be concluded that the subinhibitory concentration of hydrogen peroxide can slow down but does not inhibit the biofilm formation of *E. coli*. **We have also observed that during the incubation with subinhibitory concentration of hydrogen peroxide, the production of cellulose was significantly increased in case of clinical and reference No. 71.8 of *E. coli* strains and its isogenic mutant csgBA::Cm with inhibited expression of 'curli' and with the active production of cellulose.** The literature states that there are common stages in the cellulose and curli expression pathways – *csgD* is a regulator of curli transcription and it also activates the cellulose biosynthesis (Gerven et al., 2018). However, we have observed a reduction in the expression of *csgBA* and *csgD* in the wild type of *E. coli* strain under the treatment with hydrogen peroxide (unpublished data), which indicates another independent expression of cellulose induced by hydrogen peroxide, however, these observations require further investigation. **Although the literature describes a positive effect of cellulose on biofilm formation, our results did not confirm this statement - the increased cellulose production did not correlate with the increased**

biofilm formation. Our results suggest that cellulose production may protect bacteria against the toxic effects of oxygen free radicals and other components of the inflammatory process, as mentioned by other authors (Zhou et al., 2001; Kai-Larsen et al. ., 2010). **It should also be considered that cellulose production may modify the adhesive properties of bacterial surfaces (Krasowska and Sigler, 2014; Gonçalves et al., 2016). If the presence of free radicals induces increased cellulose production, then bacteria can become more hydrophilic and can increase affinity for hydrophilic surfaces (Ma and Wood, 2009; Adamus-Białek et al. 2015).** Recent studies have shown that the use of hydrophilic materials in catheters may prevent UTI (Cardenas and Hoffman, 2009, Rognoni and Tarricone, 2017). On the other hand, the affinity of cellulose-producing strains for hydrophilic surfaces may increase the risk of infection in catheterised patients. Certainly, cellulose production and biofilm formation increase the bacteria's ability to infect the urinary tract, often recurrent (Norinder et al., 2011). It should also be considered that the cellulose production induced by free radicals reduces the adhesion to the mucous membrane, which leads to easier elimination from the urinary tract. In addition, the bacterial response to ROI is associated with the expression of many genes, including genes encoding other virulence factors. The contact of uropathogenic *E. coli* with eukaryotic cells triggers a cascade of complicated events (Blomgran et al., 2004). Exposure of *E. coli* to ROI activates a diverse set of antioxidant genes that are controlled by the SoxRS regulator, endonuclease IV, and glucose-6-phosphate dehydrogenase. H_2O_2 also induces an OxyR transcription activator that controls gene expression for catalase/hydroperoxidase (katG), glutathione reductase (gorA), and C-alkyl reductase (ahpC) subunit in *E. coli* (Long et al., 1999). We observed that the logarithmic growth phase was delayed in culture with subinhibitory concentrations of hydrogen peroxide compared to growth under optimal conditions, which may represent an adaptation period in which pathogens repair intracellular damage and catalase expression to neutralise toxic H_2O_2. Uropathogenic *E. coli* are well-equipped with a whole array of mechanisms that increase resistance to oxidative and nitroactive effector molecules of the host (Fux et al., 2005). In summary, the results of our research are another proof of an uninterrupted ‘arms race’ between pathogenic bacteria and people. **In our study we proved that the subinhibitory concentrations of hydrogen peroxide are cytotoxic to the epithelial cells and are able to slow down the growth of bacteria and then the biofilm formation, but do not destroy all bacterial cells in the population. On the other hand, it has been detected that cellulose production is stimulated by hydrogen peroxide, a factor that can hinder bacterial stay in the bladder and reduce the hydrophobicity of the bacterial surface.** However, further studies on the expression of individual genes involved in this biochemical pathway, especially *csgD,* can give an important observation for the explanation to the mechanism of expression of these factors induced by hydrogen peroxide. It is also interesting how other urospecific genes encoding adhesins and toxins (e.g. cnf1, haemolysin) will be expressed in the free oxygen radicals released during the aerobic outbreak of polymorphonuclear neutrophils. Strong support and confirmation of the obtained observations would be to carry out analogous tests in tissue cultures (e.g. T24).

The obtained results of the previous studies were the inspiration for a deeper work on the uropathogenicity of the studied *E. coli* strains. The research involved the use of uropathogenic *E. coli* strains equipped with virulence genes and sensitive to all analysed antibiotics. A technique of drug resistance induction was developed through the use of subinhibitory concentration (sub-MIC) of various antibiotics (amoxicillin, ciprofloxacin, gentamicin and tobramycin). After an approximately 20 days of passage of five different *E. coli* strains in sub-MIC of particular antibiotics – over 120 derivatives with induced resistance to a given antibiotic were selected. The *E. coli* derivatives were compared to their wild types based on their susceptibility profiles, virulence genes, biofilm formations and the fingerprint profiles of CGG-PCR products based on primers containing repeated trinucleotide sequences CGG. Further analyses of the derivatives revealed a series of interesting observations and conclusions, which were published in the work **Ciprofloxacin, amoxicillin, and aminoglycosides stimulate genetic and phenotypic changes in uropathogenic Escherichia coli strains, Adamus-Białek W*, Wawszczak M, Arabski M, Majchrzak M, Gulba M, Jarych D, Parniewski P, Głuszek S, 2019, Virulence.** Currently, bacterial resistance to antimicrobials is widely explored, including not only typical mechanisms of drug resistance, but it is also being considered in the context of complex bacterial pathogenicity (Schroeder et al, 2017). Bacterial adaptation to the environment is one of the oldest mechanisms of living cells in the world, so it can be assumed that bacteria can regulate it via different pathways of metabolism. That is why we hypothesised that antibiotics can also induce global changes in the uropathogenic *Escherichia coli* cells. Generally, we intended to investigate what the consequences would appear during long-term exposure of the UPEC strains to sub-lethal concentrations of different antibiotics. Similar investigations have been conducted for a long time on many different bacterial species (Zhanel et al., 1992; Soto et al., 2006; Goneau et al., 2015; Schroeder et al., 2017; Ranieri et al., 2008). However, the effect of antibiotics on different metabolic pathways in bacteria still needs to be clarified. The bacterial exposure on the sub-MIC of antibiotics is an important factor for emergence of bacterial drug resistance, especially since it can happen during the antibiotic treatment of a bacterial infection or during preventive use of antibiotics in animal breeding.

The first observations were the generation rate of derivatives, the changes in the drug resistance profiles and the stability of the acquired resistance among selected *E. coli* derivatives. Almost all studied *E. coli* strains acquired the drug resistance during the treatment with the sub-MIC of all used antibiotics. Only one *E. coli* strain did not generate any derivatives resistant to ciprofloxacin and it turned out to be the most stable bacterial strain. **Ciprofloxacin induced the resistance the fastest, just after the 1st day of passage, and the resistance to all fluoroquinolones was observed in all derivative strains**. The similar observation was described by Soto et al. (2006). This resistance is quickly generated by mutation in *gyrA* and *parC* (Cirz et al., 2005) that is irreversible and strongly correlated with all fluoroquinolones. This corresponds with previously presented synergistic effect of antibiotics detected with Cohen's kappa correlation, where the resistance to one fluoroquinolone was compatible with the resistance to all

fluoroquinolones (Adamus-Białek et al., 2017). **Additionally, the observed correlation between simultaneous acquisition of the ciprofloxacin-induced resistance to fluoroquinolones, amoxicillin/clavulanate and trimethoprim indicates the dangerous mechanism of cross-resistance among the UPEC strains.** That cross-resistance could be correlated with the induction of the efflux pump, which removed the antibiotics from the cell (Saito et al., 2006). Chang et al. (2007) suggested that the mutations in *gyrA* or *parC* were strongly correlated with the overexpression of the AcrAB efflux pump and resistance to betalactams, including clavulanic acid. Similar correlations of the increased expression of AcrAB/TolC and the decreased expression of OmpC in the ciprofloxacin-resistant mutant of *S. typhimurium* were presented by Fabrega et al. (2009). The role of the ArcAB-TolC in the induction of multi-drug resistance was described also by other authors (Liu et al., 2004; Marquet et al., 2018)**. We agree that the exclusion of fluoroquinolones from routine treatment in outpatient therapy and searching for alternatives should be considered.** The application of fluoroquinolones is becoming increasingly questionable and the limitation of their use is promoted by many scientists (Trautner, 2018; Durkin et al., 2018). **However, ciprofloxacin induced cross-resistance to the smallest number of antibiotics in comparison to other antibiotics. Amoxicillin also induced the resistance very rapidly (just after the 2nd day of passage) and it generated the most numerous groups of derivatives**. However, amoxicillin induced the resistance to other betalactams only in approximately 25% of derivatives, but the resistance to amoxicillin/clavulanate appeared in 85% of this group of derivatives. Considering the stability of the induced resistance, the derivatives lost the resistance to amoxicillin or cefoxitin during the passage of culture in optimal conditions, whereas the resistance to amoxicillin/clavulanate was irreversible, which was also observed in the case of ciprofloxacin-induced resistance. The induction of the overexpression of the chromosomal beta-lactamase AmpC in the case of *E. coli* is not possible because of the lack of the *ampR* gene, which is involved in the activation of *ampC* transcription. Therefore, AmpC in *E. coli* is noninducible but is controlled by the promoter and attenuator mechanisms (Jacoby, 2009). These observations could probably be a result of ArcAB overexpression as it was mentioned above. Another possibly induced mechanism might be the return to the original membrane structure with the OmpC porins expression. However, the strong conclusion concerning the technical aspects seems to play an important role for this observation. During the analyses of the diameter zones of bacterial growth inhibition, they were similar around the amoxicillin disc and the amoxicillin/clavulanic disc, and the sensitivity to these antibiotics was strongly decreased in comparison to the wild type strains. Thus, these differences can arise from the technical properties of clinical breakpoint tables from EUCAST, where the norms of classification of bacteria in terms of resistance in the case of amoxicillin and amoxicillin/clavulanic are different. Finally, only the resistance to imipenem did not appear in any derivatives. **All our previous and current studies proved that imipenem seems to be the most effective antibiotic against the UPEC strains**. Similar observations were presented by other authors (Yayan et al., 2015). Generally, betalactam antibiotics are presented as safer than others for treatment and currently they are the gold standard for antibiotic therapy. However,

the increasing emergence of carbapenem-resistant bacterial strains producing new variants of NDM betalactamase is well known (Grover et al., 2017; Rahman et al., 2018). In our study, besides the rapidly emerging antibiotic tolerance, amoxicillin induced cross resistance to the largest number of antibiotics. The routine and empirical application of betalactams is not a good solution, considering these findings and the constantly emerging new beta lactamases with an increasingly broad spectrum of their activity. During our research the least influential antibiotics turned out to be aminoglycosides, of which the rarest induce cross-resistance in the *E. coli* derivatives. These observations result from other drug resistance mechanisms that are associated with translation and the ribosomal structure (Serpersu et al., 2008). Additionally, **we also observed the statistically significant increased susceptibility to ciprofloxacin in correlation to resistance to gentamycin. It may result from their antagonistic mechanisms of drug-resistance.** Suzuki et al. (2014) proved that resistance to aminoglycosides reduces proton-motive force that decreases the AcrB expression, leading to susceptibility to drugs which bacteria cannot exclude from the cell. Interestingly, **we also observed an opposite phenomenon, wherein the induction of resistance to ciprofloxacin caused increased sensitivity to gentamycin and netilmicin. Maybe that reverse dependence can be connected with increased expression of the acrAB–TolC efflux system**. Atac et al. (2018) proved that among *E. coli* ST_{131} strains, the *marA* overexpression was correlated to the resistance to quinolones. What is more, the gentamicin resistance was statistically lower in ST_{131} than in non-ST_{131}. In our research, amoxicillin also revealed similar dependence, but only in the case of netilmicin. Generally, it is known that fluoroquinolones, betalactams and aminoglycosides can act as synergists, so this intriguing observation can be a starting point for further studies. **This is the first time the phenomenon of that antagonistic relationship between ciprofloxacin and gentamycin has been observed**. In conclusion, one antibiotic can lead to numerous cross-resistances emerging among *E. coli* strains. All four antibiotics induced the resistance to the other antibiotics from all 6 analysed classes, except ciprofloxacin, which did not induce the resistance to aminoglycosides and imipenem. As we mentioned above – it can result from various mechanism of drug resistance that could be induced by the stress-response of the *E. coli* cell. Antibiotic-induced stress inside the bacterial cell can change the gene expression profile and it regulates the bacterial metabolism, it also has an impact on other virulence properties of bacteria (Horinouchi et al., 2017; Atac et al., 2018).

The subsequent important stage of our study was to verify the extent to which the antibiotics are able to influence the virulence genes of the UPEC strains. It has been long observed that antibiotic resistance correlates with certain bacterial features, this correlation is especially observed between fluoroquinolones and bacterial virulence (Martinez-Martinez et al., 1999, Soto et al. 2006; Da Silva and Mendonça, 2012). In our study, the presence of 6 virulence factor gene regions (*papC*, *sfaE/D*, *cnf1*, *usp*, *fimG/H*, *hlyA*) and their expression were analysed. These genes (except *fimG/H*) are described in the literature as specific for the UPEC strains (Yuri et al., 1998). It is interesting that the presence of specific virulence-associated genes and deep comprehensive phylogenetic analysis distinguishes UPEC from many commensals and intestinal pathogenic *E. coli* strains (Dobrindt et al, 2003; Elena et al., 2005;

Brzuszkiewicz et al., 2006). Most of these urovirulence genes are carried on the Pathogenic Islands (PAIs) [Kao et al., 1997; Dobrindt et al., 2002; Kurazono et al., 2003]. One analysed gene-fragment – *fimG/H*, encodes subunits of Fimbria Type I. That fimbria is crucial for bacterial adhesion at the early stage of UTI, but it is very commonly present for all type of *E. coli* strains and it is chromosomally encoded (Bahrani-Mougeot et al, 2002). Its stable position in the *E. coli* genome seems to be important to ensure the primary adhesive property. **This was probably the reason why *fimG/H* were preserved in all derivative strains in our study and that fimbria is rather like a fitness, not a virulence factor** [Dobrindt et al., 2003]. In case of the urovirulence factor genes – only ciprofloxacin induced the loss of these genes. The mentioned above one *E. coli* strain did not acquire the resistance to ciprofloxacin, which was equivalent with conservation of all virulence factor genes. This could mean that the presence of these genes was stabilised and the mobility properties of the PAIs were lost. In case of other *E. coli* strains – the loss of virulence genes appeared just after the first day of passage, yet generally the strains were losing their factors in various ways. It could be connected with the presence of studied genes on various PAIs, diverse equipment of mobile sequences or DNA repair systems efficiency in the bacterial genome. Although the correlation between the presence of the urovirulence genes and antimicrobial resistance susceptibility was often described, the effects of long-time pressure of sublethal antibiotics concentrations on the UPEC strains has not been clarified. Some sources indicate that DNA repair mechanisms are related to this phenomenon. A similar study was presented by Soto et al. (2006), they also observed the simultaneous loss of *hly* and *cnf1* in all studied UPEC strains just after the first day of passage. In opposed to our results, they did not observe the loss of *pap* and *sfa* genes. Sanchez-Cespedes et al. (2015) observed that *gyrA* mutation decreased the expression of *fimA, papA, papB* and *ompA*. This mechanism is probably related to a change of DNA topology, which disrupted the normal gene expression process. The gyrase expression plays an important role here, which can relax the DNA helix and lead to mutational changes in bacterial genome via DNA-repair systems (Hsu et al., 2006). However, the observed phenomenon (Goneau et al., 2015; Sanchez-Cespedes et al., 2015) indicates that SOS activation is not necessary for the loss of virulence factor genes induced by ciprofloxacin. Perhaps the observed duality of the results arises from other mechanisms of DNA repair, such as the Double-Strand break repair, mismatch repair or antibiotic-induced competence for transformation in response to stress (Zgur-Bertok et al., 2013; Schroeder et al, 2017). Maybe it is worth to consider that a different DNA sequence of mobile elements of different PAIs can be of significance to this study.

Further analyses included the antibiotic influence on biofilm formation. Although it has been frequently described, these observations concerned analysis in real time of bacterial incubation with antibiotic (Ranieri et al., 2018). **We present for the first time how an antibiotic can permanently change the ability to form biofilm among the UPEC strains**. Only amoxicillin-induced derivatives of *E. coli* strains demonstrated a statistically significant higher level of biofilm formation and, what is important, it was not dependent on the density of planktonic cells. What should be emphasised – the wild types of the analysed *E. coli* strains exhibited a very low level of biofilm formation, whilst after

the treatment with amoxicillin – their selected derivative strains demonstrated up to 4-times stronger biofilm. This may indicate that biofilm formation can be induced by amoxicillin even in the strains unable to create biofilm. This situation can be very adverse during UTI treatment, where a sublethal concentration of amoxicillin in the urinary tract can lead not only to the selection of resistant cells but also facilitate bacterial adhesion to the uroepithelial tissue of the host and help develop bacterial biofilm. Amoxicillin belongs to antibiotics that affect the bacterial cell wall structure and induce bacterial stress, which can stimulate biofilm formation (Kaplan, 2011). The changes of bacterial cell surface can have an effect on their hydrophobicity and consequently on biofilm formation. Similar extensive studies have been carried out by Goneau et al. (2015). They described an *in vivo* study, in which the subinhibitory antibiotics (ciprofloxacin, ampicillin and gentamicin) modulated the virulence in the uropathogens, inter alia the *Escherichia coli*. The induction of the expression of adhesins caused an increase in biofilm formation, colonization of the murine bladder and the kidneys, and promoted intracellular bacterial community. A similar observation *in vitro* in other bacterial species has also been described in the literature (Erdeljan et al., 2012; Mlynek et al., 2016). Taking our results into account - the growth curves of derivatives were not disturbed, so the increase of biofilm formation did not result from the increase of lysed bacterial cells. Mlynek et al. (2016) suggest that the amoxicillin stimulates extracellular DNA-dependent biofilm formation in bacteria, which can reflect an adaptation to the cell wall stress. **To sum up, other authors showed the increase of biofilm during treatment with betalactams, while our results present a stable increase of biofilm formation after the treatment with amoxicillin. Furthermore, the derivative strains continued the increase of biofilm formation in subsequent days of passage without amoxicillin, which can suggest the induction of some specific gene expressions and their following overexpressions**.

On the last stage of the study we genotyped the derivatives and their wild types of the studied *E. coli* strains via the CGG-PCR developed in our previous study (Adamus-Białek et al., 2009). This technique maps the genomic fingerprints of the studied strains. In the previous study, the designed CGG-PCR have divided the studied *E. coli* strains into two groups with different pathogenicity. On the other hand, the CGG-PCR indicated the subtle differences specific to individual strains. Following these achievements, we wanted to look into the genomes of *E. coli* derivatives using the same method. The primary band patterns of the CGG-PCR products were preserved in the *E. coli* derivatives compared to their wild type strains, but the differences were also observed by disclosure or disappearance of single bands. These differences corresponded the most with the ciprofloxacin-induced derivatives, especially where the loss of virulence factor genes was also observed. However, slight differences were also observed after the treatment with amoxicillin. These findings are justifiable because of the strong influence of ciprofloxacin on DNA and its metabolism (Hsu et al., 2006; Soto et al., 2006; Goneau et al., 2015). Amoxicillin has a weaker impact, but its ability to induce free radicals can affect DNA (Miller et al., 2004). **The stable band patterns of aminoglycoside-induced derivatives result from the lack of an aminoglycoside influence on the DNA structure**. It is worth to add that observed changes of the

CGG-PCR band patterns were induced by antibiotics because no changes were observed after the passages of the culture in optimal conditions. The method of genotyping via MLEE or ribotyping of the rDNA via RFLP are the standards used for bacterial differentiation, however, they require extensive laboratory experience and they are unable to differentiate the bacterial pathogenicity (Silveira et al., 2001). These results confirm the previous conclusion that the CGG-PCR may be a useful technique for epidemiological investigation of kinship between *E. coli* strains.

To summarise, our study provides a broad description of the correlation between sublethal antibiotic treatment and changes of drug resistance profiles, cross-resistance acquisition or antagonistic effects between antibiotics. Additionally, the antibiotic influence on the virulence factor gene loss and the increase of biofilm formation among UPEC strains was proven. The greatest and the most stable changes were observed in the case of ciprofloxacin, which confirms that ciprofloxacin has a strong influence on the DNA metabolism and/or activates other pathways which bacteria use for adaptation in unfavourable environment. The bactericidal effect lasts the longest in the case of aminoglycosides. They induce the least changes in bacteria, which exhibit the highest sensitivity to them. Unfortunately, aminoglycosides cause the most numerous and the strongest side effects in human in comparison to fluoroquinolones and betalactams. Our study exhibits the multiplex effect of antibiotics on the UPEC strains. This group of pathogens exhibit a high capacity to resist modern therapies. They are often responsible for frequent recurrent infections as well as fast drug resistance build-up (Durkin et al., 2018). The ability to form an intracellular biofilm is a way to cause permanent presence in the urinary tracts of infected patients, which can lead to severe damage or destruction of this system. The understanding of antibiotic resistance mechanisms of the UPEC strains is a chance to develop better therapies. This can lead both to health-related and economic benefits and the presented results are an important signal to reflect on the restrictive use of antibiotics.

4.3.3. SUMMARY AND FUTURE SCIENTIFIC OBJECTIVES

The scope of research presented as scientific achievement concerned a detailed analysis of *Escherichia coli* strains isolated from the urine of patients diagnosed with urinary tract infection. In my opinion, defining the studied collection of *E. coli* strains as uropathogenic strains is inadequate. Uropathogenicity is the ability of bacteria to infect the urinary tract, however, many factors predisposing to infection independent of bacteria are known. The obtained results suggest that the tested group of *E. coli* strains is a heterogeneous group of bacteria, showing different properties in terms of antibiotic resistance, biofilm capacity and the presence of specific genes.

The studies on *E. coli* strains isolated from the urine of patients diagnosed with UTI included:

- An innovative approach used in a detailed analysis of drug resistance and the study of complex correlations using advanced statistical and mathematical tools.
- The study of *E. coli* ability to survive under unfavourable environmental conditions: urine, free radicals, subinhibitory concentration of antibiotics.
- The role of biofilm and cellulose production in the process of adaptation to toxic environmental conditions: urine, free radicals.
- The determination of the effect of ciprofloxacin, amoxicillin and aminoglycosides on the genetic and phenotypic variability of uropathogenic *E. coli* strains.

As the most important achievements in the presented cycle of publications I consider following conclusions:

- the studied collection of *E. coli* strains isolated from the urine is a heterogeneous group of bacteria showing enormous differences in drug resistance and virulence profiles and biofilm capacity,
- the application of advanced mathematical tools in the study of deep dependencies between the antibiotics and bacterial strains in the analysis of drug resistance has a high potential for application,
- the application of mathematical analyses can be used in rational antibiotic therapy and epidemiological studies,
- the presence of the analysed resistance genes does not play the role of a phenotypic marker of resistance to betalactam antibiotics, aminoglycosides and sulfonamides,
- analysis of mutations in the *gyrA* and *parC* genes can be a predictor for the monitoring of drug resistance to fluoroquinolone antibiotics,
- accumulation of nonspecific mutations in the *gyrA* and *parC* genes decrease the sensitivity to fluoroquinolones

- the growth and biofilm formation of *E. coli* are weaker in the urine or free radicals' conditions compared to the optimal conditions,
- *E. coli* is resistant to high concentrations (~ 0.3 M) of hydrogen peroxide, which may imply that the neutrophil aerobic blast does not have a bactericidal effect,
- under unfavourable environmental conditions (urine, free radicals) *E. coli* strains are more involved in biofilm formation than in cell division (growth),
- free radicals (released during the outbreak of aerobic neutrophils) stimulate *E. coli* cells to produce cellulose, which may hinder the treatment of catheterised patients due to the increased strength of bacterial adhesion to the surface of the catheter,
- ciprofloxacin is not a safe antibiotic in the treatment of UTI due to the rapid mutagenic effect, the induction of cross-resistance and the changes in the virulence profile of UPEC strains,
- amoxicillin is not a safe antibiotic in the treatment of UTI, due to the induction of cross-resistance to many antibiotics and stimulation to increased biofilm formation among UPEC strains,
- ciprofloxacin, aminoglycosides and amoxicillin exhibit an antagonistic effect.

In the future, I intend to continue research on the uropathogenicity of *E. coli* strains. The research topic that I am planning to develop is the effect of antibiotics, mainly fluoroquinolones, on the instability of the CRISPR/cas sequence. The CRISPR/cas sequences act as a specific defence mechanism of the bacteria against foreign DNA, mainly viral. Reports indicate that there is a strong association between the presence of CRISPR sequences and reduced resistance to antibiotics, which gives grounds to hypothesise that the presence of CRISPR sequences reduces the adaptive potential of bacteria. Our preliminary studies revealed that in the case of *E. coli* mutants with induced ciprofloxacin resistance the CRISPR regions have been changed as well. The results of these studies 'The Factors that influence the genetic stability of CRISPR/cas systems in *Escherichia coli'* were presented at the conference 'Microbiology in Health Care and Environmental Protection' – MICROBIOT in 2017 in Łódź. I assumed that antibiotics can induce changes in these regions, which has consequences in changed virulence and adaptation properties of *E. coli* bacteria. I am going to explain this hypothesis in further research with the intention of submitting Sonata Bis grant application to the National Science Centre.

Scientific research on the genetic determinants of metabolic diseases, mainly pancreas in humans, became my additional scientific interest. When I started the work in the Department of Surgery and Surgical Nursing in The Faculty of Medicine and Health Sciences Jan Kochanowski University I have been involved in the research conducted for many years by Professor dr hab. Stanisław Głuszka and His Team. Access to the wide clinical material (over 1256 samples of clinical material taken from healthy people and with pancreas diseases) is an excellent source for broadening scientific horizons and research capabilities. In connection with the beginning of new directions of research, I became the auxiliary

supervisor of the doctoral thesis, where we search for new genetic markers of pancreatitis, with particular emphasis on the role of the carboxypeptidase 1 gene. I also took part in research on the genetic determinants of the Metabolic Team, and the first effects of cooperation were presented in the work of Suliga E., Kozieł D., Cieśla E., Rębak D., Wawszczak M., Adamus-Białek W., Naszydłowska E., Piechowska A., Głuszek S., Omentin rs2274907 gene polymorphism and the risk of metabolic syndrome: a preliminary report, Medical Studies, 2018; 34 (4): 267-275. The results of the obtained research works were also presented at scientific conferences in 2018. My microbiology experience also gave me the opportunity to act as the auxiliary promoter of another PhD student, who conducts a research into the factors determining the occurrence of *Listeria* spp. in hospitals and social welfare homes. I hope that it will be another branch of a new, fascinating scientific cooperation.

4.3.4. LITERATURE

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5. Description of other scientific and research achievements

5.1. Research topics implemented

In addition to the presented cycle of publications, specified in the scientific achievement, I have co-authored 12 original publications, published in peer-reviewed journals with a total of 10,213 Impact Factor points (136 MNiSzW). In the case of 6 works, I am the first and correspondent * author. I am also a co-author of 28 conference reports on national, international or foreign scale. The obtained results of the conducted research were divided into five thematic areas, to which appropriate publications were assigned:

I. The use of TRS sequences in genotyping and bacterial epidemiological analysis

1. Adamus-Bialek W*., Wojtasik A., Majchrzak M., Sosnowski M., Parniewski P., (CGG)4-based PCR as a Novel Tool for Uropathogenic Escherichia coli Discrimination: Comparison with ERIC-PCR, Journal of Clinical Microbiology, 2009, 47(12):3937-44,

IF: 4,162; MNiSzW 24

My contribution to this publication concerned the participation in the development of the concept of work, the optimization and performance of the experiments, the description of procedures in the materials and methods section, the analysis and interpretation of the research results, the preparation of the graphic part of the presented results, the preparation of the manuscript for the publication, the improvement of the entire article in response to reviews and the correspondence with the editors. Overall, I estimate my participation at 50%.

2. Wojtasik A., Majchrzak M., Adamus-Bialek W., Augustynowicz-Kopec E., Zwolska Z., Dziadek J., Parniewski P., Trinucleotide repeat sequence-based PCR as a potential approach for genotyping Mycobacterium gordonae strains., Journal of Microbiological Methods, 2011, 85(1):28-32,

IF: 2,086, MNiSzW 25

My contribution to this publication concerned the participation in the development of the concept of work, the optimization of the experiments (TRS-PCR method) and the interpretation of the research results. I estimate my contribution at 10%.

II. Application of FTIR spectroscopy and artificial neural networks in genetic and phenotypic analysis of bacteria

3. Lechowicz L., Adamus-Bialek W., Kaca W.; Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy and Artificial Neural Networks Applied to Differentiate Escherichia coli papG+/papG- strains., Journal of Spectroscopy, 2013, (2013), Article ID 538686, 3 pages, http://dx.doi.org/10.1155/2013/538686

IF: 0, MNiSzW 0

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (the preparation of the research material, PCR reaction specific to the papC gene) and the interpretation of the test results. I estimate my contribution at 25%.

4. LechowiczL., Urbaniak M., Adamus-Bialek W., Kaca W., The use of infrared spectroscopy and artificial neural networks for detection of uropathogenic Escherichia coli strains' susceptibility to cephalothin., Acta Biochimica Polonica, 2013, 60(4): 713-718

IF 1,389, MNiSzW 15

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (the preparation of the research material) and the interpretation of the research results. I estimate my contribution at 25%.

III. Sanitary analysis of selected environments

5. Adamus-Bialek W.*, Karwacka K., Bak L., Microflora of the selected water reservoirs in Swietokrzyskie Voivodship, Acta Biochimica Polonica, 2013, 60(4): 689-693

IF 1,389, MNiSzW 15

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (planning the experimental work and the participation in their implementation), the interpretation of the research results, the preparation of the manuscript and the correspondence during the review of the manuscript. I estimate my contribution at 65%.

6. Adamus-Białek W.*, Wawszczak M., Filipiak A., Woźniak A., Jasek P., Głuszek S., Sanitary state of surface waters in Świętokrzyskie voivodeship, Medical Studies, 2019, 35(1):10–15

IF 0, MNiSzW 10

My contribution to this publication concerned the creation of the work concept, the experimental part (planning the experimental work and the participation in their implementation), the interpretation of the research results, the preparation of the manuscript and the correspondence during the review of the manuscript. I estimate my contribution at 70%.

7. Adamus-Białek W.*, Wawszczak M., 16S rRNA sequences used to identification of bacterial DNA isolated from various materials, Rocznik Świętokrzyski seria B – nauki przyrodnicze;2014;35;3-14

IF 0, MNiSzW 1

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (planning the experimental work and the participation in their implementation), interpretation of research results, participation in the preparation of the manuscript and the correspondence during the review of the manuscript. I estimate my contribution at 70%.

8. Adamus-Białek W.*, Jóźwiak M., Wawszczak M., Microbiological air pollution in central part of Swietokrzyskie Mountains, Rocznik Świętokrzyski Seria B – nauki przyrodnicze; 2014; 35; 13-25;

IF 0, MNiSzW 1

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (planning the experimental work and the participation in their implementation), the interpretation of research results, the participation in the preparation of the manuscript and the correspondence during the review of the manuscript. I estimate my contribution at 70%.

9. Adamus-Białek W.*, Wawszczak M., Świercz A., Impact of sewage treatment plant on local environment; Proceeding of Ecopole, 2015, 9(2): 4-7

IF 0, MNiSzW 9

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (planning the experimental work and the participation in their implementation), the interpretation of the research results, the participation in the preparation of the manuscript and the correspondence during the review of the manuscript. I estimate my contribution at 70%.

10. Adamus-Bialek W.*, Wawszczak M., Microbiological contamination of food, Ecol Chem Eng A, 2015, 22(4):509-516

IF 0, MNiSzW 6

My contribution to this publication concerned the participation in the development of the concept of work, the experimental part (planning the experimental work and the participation in their implementation), the interpretation of the research results, the participation in the preparation of the manuscript and the correspondence during the review of the manuscript. I estimate my contribution at 70%.

IV. Study of the effect of heavy metal complexes on the production of pyoverdine and P. aeruginosa

11. Gałczyńska K., Kurdziel K., Adamus-Białek W., Wąsik S., Szary K., Drabik M., Węgierek-Ciuk A., Lankoff A., Arabski M., The effects of nickel(II) complexes with imidazole derivatives on pyocyanin and pyoverdine production by Pseudomonas aeruginosa strains isolated from cystic fibrosis; Acta Biochimica Polonica, 2015, 62(4): 739-745

IF 1,187, MNiSzW 15

My contribution to this publication concerned the participation in the experimental part (the analysis of the effects of the tested compounds on P. aeruginosa strains), the analysis and interpretation of the results and the participation in the preparation of the manuscript. I estimate my contribution at 20%.

V. Genetic analysis of human metabolic diseases

12. Suliga E., Kozieł D., Cieśla E., Rębak D., Wawszczak M., Adamus-Białek W., Naszydłowska E., Piechowska A., Głuszek S., Omentin rs2274907 gene polymorphism and the risk of metabolic syndrome: a preliminary report, Medical Studies, 2018; 34(4): 267–275

IF 0, MNiSzW 10

My contribution to this publication concerned the participation in the planning and executing the laboratory tests and the interpretation of the research results. My estimated contribution is 10%.

I started to be interested in the bacterial genetics during my M.Sc. studies in biology at the Świętokrzyska Academy in Kielce. Implementing the master's thesis entitled 'Analysis of the transposon mutants of *Proteus mirabilis* S1959 in reactions with anti-O3 and anti-R110 sera' in the Department of Microbiology had the opportunity to learn many techniques of both molecular biology and microbiology. The mutants of *P. mirabilis* were obtained during the transposon mutagenesis and after that they were analysed based on the swarming growth and in reaction with anti-O3 and anti-R110 sera using the ELISA test. I found out how interesting work with bacteria can be. Knowledge and skills gained during this period inspired me to continue my scientific development even further. I was particularly interested in the adaptive mechanisms of bacteria that allow them to survive and live in adverse environmental conditions. I started Ph.D. studies at the University of Lodz and I conducted experimental research at the Laboratory of Molecular Genetics at the Polish Academy of Sciences in Łódź under the supervision of dr hab. Paweł Parniewski. The entourage of eminent scientists gave me the opportunity to profit from their knowledge and skills at molecular biology. At the time I was inspired by the pathogenicity mechanisms of *Escherichia coli* infecting the urinary system, which was the original purpose of my doctoral thesis. I collected 127 clinical strains of *E. coli* isolated from the urine of patients diagnosed with urinary tract infection. Next, I developed and performed a multiplex-PCR analysis for the presence of 6 selected genes coding for specific pathogenicity factors. Based on the literature data, the virulence factors were selected based on the uropatogenicity: Type 1 fimbriae, Fimbria P, Fimbria S, type 1 necrosis factor cytosis, haemolysin α, bacteriocin. The presence of the studied virulence genes was correlated with drug resistance. A statistically significant relationship was found between the occurrence of the studied genes and the sensitivity to fluoroquinolones. A similar correlation of pathogenicity factors with drug resistance was previously observed in *E. coli* (Martinez-Martinez et al., 1999, Vila et al., 2002, Horcajada et al., 2005). The main achievement of my doctoral thesis was the development of a genotyping technique for *E. coli* strains based on the trinucleotide repeats (CGG)n. It was an innovative method that gave, so far, the highest degree of repeatability of obtained results and allows for very wide differentiation of bacteria – it includes deep clonal genetic differences between the individual isolates. However, most importantly, the newly developed technique differentiates the strains into two separate biological groups – group I of strains with high pathogenicity potential (presence of virulence genes) and susceptibilities to fluoroquinolones, group II of strains with

low pathogenicity potential (no virulence genes) and high resistance to fluoroquinolones. The results of these studies were published at work **(CGG)4-based PCR as a Novel Tool for Uropathogenic Escherichia coli Discrimination: Comparison with ERIC-PCR, Adamus-Bialek W*., Wojtasik A., Majchrzak M., Sosnowski M., Parniewski P., Journal of Clinical Microbiology, 2009, 47(12):3937-44.** These studies introduced a new technique of bacterial genotyping to molecular biology. The results have proven that the trinucleotide repeat sequences of E. coli strains are associated with their pathogenicity. It can be concluded that the TRS sequence instability is linked to DNA repair mechanisms such as the SOS mechanism that is induced by fluoroquinolones (Soto et al., 2006). The universality of this method in the genotyping of pathogenic bacteria has been proven in the next work **Trinucleotide repeat sequence-based PCR as a potential approach for genotyping Mycobacterium gordonae strains, Wojtasik A., Majchrzak M., Adamus-Bialek W., Augustynowicz-Kopec E., Zwolska Z., Dziadek J., Parniewski P., Journal of Microbiological Methods, 2011, 85(1):28-32.** In this case, another TRS - $(CAC)_4$ sequence turned out to be useful in the rapid and deep clonal analysis of the difficult to diagnose non-tuberous *M. gordonae* mycobacteria, which also have significant clinical and epidemiological significance. The newly developed TRS-PCR genotyping technique utilizing various trinucleotide repeat sequences is a promising tool in epidemiological research. A feature of this technique is the high index of strain differentiation and over 90% of the reproducibility of the obtained genetic profiles, which distinguishes it from other genotyping methods also based on repetitive sequences such as ERIC-PCR.

The primary ground of the search for new and alternative techniques for differentiation of microorganisms is to reduce costs and time spent on analysis. It also aims to quickly and easily identify clinically important microorganisms or their properties. After developing a new bacterial genotyping technique based on TRS sequences, I was invited to cooperate with the Scientific Team of prof. dr hab. Wiesław Kaca (Head of the Department of Microbiology, UJK). We adopted advanced technique of attenuated infrared spectroscopy with Fourier transform ATR FT-IR in genetic and phenotypic analysis of bacteria. The artificial neural networks were developed to read complex IR spectra of bacteria in correlation with their properties. The article **The use of infrared spectroscopy and artificial neural networks for detection of uropathogenic Escherichia coli strains' susceptibility to cephalothin, LechowiczL., Urbaniak M., Adamus-Bialek W., Kaca W., Acta Biochimica Polonica, 60(4):713-718 2013** describes for the first time the application of this technique in microbiological analyses. The research material was the collection of uropathogenic strains of *Escherichia coli*, which I used in previous studies. The IR spectra specific for each strain were correlated with the sensitivity to cephalothin. It has been surprising that the obtained bacterial IR spectra can be further interpreted in the search for correlation with other bacterial properties. The novel technique turned out useful in the genetic differentiation of *E. coli* strains tested. The association between the individual IR spectra and the presence or absence of the papG gene was detected with the accuracy of over 80% in the studied *E. coli* strains. The results were published in **Attenuated Total Reflectance Fourier Transform Infrared**

Spectroscopy and Artificial Neural Networks Applied to Differentiate Escherichia coli papG+/papG- strains, Lechowicz L. Adamus-Bialek W., Kaca W., Journal of Spectroscopy, 2013, (2013), Article ID 538686, 3 pages, http://dx.doi.org/10.1155/2013/538686. It is not explained which molecules and how they are used for the interpretation of bacterial spectra. This is probably a general molecular profile but further analyses are needed to explain the specificity of chemical components correlating with individual properties of bacteria. The fast and simple ATR FT-IR technique creates new diagnostic possibilities but further research is necessary to create a reference database and tools for routine interpretation of the obtained bacterial spectra.

Within my microbiological research, bacterial interactions with the environment and factors that may influence their occurrence were further inspiration for my scientific development. A long-term work at the Department of Environmental Protection and Modelling of the Faculty of Mathematics and Natural Sciences of the Jan Kochanowski University has contributed to broadening my scientific interests with an epidemiological aspect in environmental research. I conducted a series of analyses on a local scale, which considered the presence of bacteria in various environments, such as water reservoirs, bottom sediments, air, food products, sewage treatment plants. One of the most important environmental problems is the inadequate quality of surface waters in the world, which is most affected by the economic and environmental factors of a given territory (Mazari-Hiriart et al., 2008, Hlavsa et al. 2011). Many organizations, both around the world and in Poland, are involved in the protection and control of water resources. However, with current trends, more than half a billion people do not have an access to adequate water quality. Every year, the contaminated water combined with the lack of access to basic sanitary conditions contributes to the death of at least 1.6 million children under the age of five (Joint Monitoring Program for Water Supply and Sanitation, 2006). Poland is among countries with insufficient water resources, which are characterised by significant pollution, seasonal variability and uneven territorial distribution (Central Statistical Office, 2013). The basic and often the only one analysis performed in sanitary and epidemiological stations is the assessment of the occurrence of faecal microorganisms, mainly *Escherichia coli* and *Enterococcus* sp. (Toranzos and McFeters, 1998; Bartram & Rees, 2000; Jones et al., 2002; Pickup et al., 2003). Unfortunately, there is a high risk of many other dangerous pathogenic bacterial such as pathogenic *E. coli* strains (VTEC, EHEC), *Salmonella* spp., *Shigella* sp., *Clostridium perfringens*, *Listeria monocytogenes*, *Vibrio cholerae* (Mazari-Hiriart et al. 2001, Le Dantec et al., 2002, Smylla et al., 2003, Hlavsa et al., 2011, ECDC, 2012, Jones et al., 2002, ECDC, 2012). In consideration to above, the cooperation with dr Łukasz Bąk from the Kielce University of Technology was initiated for detailed analysis of selected water reservoirs of the Świętokrzyskie Province. My research included the analysis of the presence of microbiological indicators (total number of mesophilic and psychrophilic bacteria, coliforms, *Escherichia coli*, *Salmonella* spp., *Shigella* sp., *Enterococcus faecalis*, *Clostridium perfringens*) in the water and bottom sediment of the selected reservoirs. The results were published in **Microflora of the selected water reservoirs in Swietokrzyskie Voivodship, Adamus-Bialek W.*, Karwacka K., Bak L., Acta Biochimica**

Polonica, 2013, 60(4):689-693. There was a large variation in the total number of bacteria and no correlation with other parameters of the tested reservoirs or environmental conditions. In water and bottom sediments of each reservoir all studied bacteria were detected (in winter and/or spring). In some cases, the values exceeded 50 cfu/ml in water or 50 cfu/g in bottom sediment. The presence of the *Shigella* sp. and *Salmonella* spp. has been confirmed in almost all water reservoirs. It should be emphasised that these values were obtained from water samples also in winter, so it can be expected that the number of bacteria will increase simultaneously with temperature, especially in summer. Furthermore, the obtained results indicate that the bottom sediment can be a reservoir of dangerous pathogens. Especially disturbing fact is that during the summer these water reservoirs are being used as recreational places, but the communal institutions are not obliged to monitor them because these reservoirs do not meet the infrastructure requirements that would classify them as recreational reservoirs according to EU standards. The similar studies were published in our next article **Sanitary state of surface waters in Świętokrzyskie voivodeship, Adamus-Białek W.*, Wawszczak M., Filipiak A., Woźniak A., Głuszek S., Medical Studies, 2019, 35(1): 10-15.** The research sites included other 3 rivers and 2 water reservoirs located in Świętokrzyskie voivodeship. The water samples were tested based on the presence of coliform, *E. coli*, mesophilic and psychrophilic bacteria. The presence of *E. coli* was confirmed in all water reservoirs, but the exceeded values was confirmed only in two cases (Wisła and Emerald Lake). The pathogens present in water can infect people and consequently they can become carriers of e.g. salmonellosis and will pose a dangerous epidemiological link. Epidemiological data still reveals frequent occurrence of salmonellosis and shigellosis in many European countries. The conducted own research is another proof that the environment is a perfect reservoir of pathogenic bacteria, and the community should be more aware of the threats and possibilities of protection against dangerous of pathogens from natural environment.

The teflon filter inserts assimilating the air and samples of activated sludge (dry and wet) were other materials for searching of an environment bacteria. The teflon filter inserts were collected from a height of 30 m above the level of mountains in the Base Station 'The Holy Cross' in the Świętokrzyski National Park. The results of the study were published in **Application of the 16S rRNA sequence in the identification of bacterial DNA isolated from various materials, Adamus-Białek W.*, Wawszczak M., Rocznik Świętokrzyski seria B – nauki przyrodnicze, 2014, 35:3-14.** The isolated total DNA was analysed by PCR to detect 16S rRNA sequences specific for bacteria belonging to *Archaea* or *Eubacteria* (Macrae, 2000). The presence of bacteria belonging only to *Eubacteria* has been confirmed. The absence of *Archaea* specific DNA may have resulted from the inadequacy of the DNA isolation technique, due to the complexity of the cell wall of bacteria belonging to this taxonomic domain. It can be concluded that the filters used for the analysis of air dust and the samples of activated sludge are good materials for further metagenomic analyses and for the searching of microorganisms with unique properties. Further research focused on the phenotypic analysis of the bacteria isolated from the filters. They were incubated under different growth conditions and on different medium, followed

by macroscopic, microscopic and nutritional requirements observations. Differential culture conditions allowed to isolate various bacterial colonies. Selected bacteria were used to prepare coloured microscopic specimens using the Gram-staining method. In addition, the biochemical properties of isolated microorganisms were analysed. The obtained results were published in **Microbiological air pollution in the central part of the Świętokrzyskie Mountains, Adamus-Białek W.*, Jóźwiak M., Wawszczak M., Rocznik Świętokrzyski Seria B – nauki przyrodnicze, 2014, 35:13-25.** Based on the macroscopic observation of single colonies, many different strains were observed, despite the repeated cell forms observed under the microscope. Most of them were mesophilic heterotrophic bacteria. The presented results show the potential use of air filters in search for microorganisms with characteristic adaptive properties. The source of the analysed dust transmitted by the air movements can be power plants, industrial plants, heat plants, communication and households. A significant impact on the condition of the atmospheric air in the Świętokrzyskie region are also distant urban-industrial centres (Upper Silesian Industrial District GOP, the region of Moravian Ostrava in the Czech Republic). These sites develop specific conditions, often with extreme parameters, which determines the occurrence of microorganisms with unique metabolic properties. The presented work is innovative because the filter samples for microbiological tests were never taken from the tested area. A specific factor is also the height - 30 m from the ground above the branches of trees (595 m above sea level) from which the air dust was absorbed into the research filter. However, it should be emphasised that the work presents very general and basic results, which may be an interesting introduction to further research in the future.

Continuing the epidemiological research, I started cooperation with the sanitary and epidemiological stations in the Świętokrzyskie Province. The available data of the distribution and contamination of sewage sludge which was enriched by our analysis of air around the sewage treatment plants gave many interesting observations and conclusions. The results of the study were published in the work **Impact of sewage treatment plant on local environment, Adamus-Białek W.*, Wawszczak M., Świercz A., Proceeding of Ecopole, 2015, 9(2):4-7.** The aim of the study was to analyse three sewage treatment plants (in Busko-Zdrój, Kazimierza Wielka and Pińczów, in the Świętokrzyskie Voivodeship) in terms of sanitary status of sewage sludge (occurrence of *Salmonella* spp. and intestinal parasitic eggs) and their management. The microbiological quality of sewage sludge, mineral composition and soil organic matter (humus) was sufficient enough to use them as natural fertilisers, but not for the cultivation of plants intended for human consumption due to the presence of *Salmonella* spp. and/or pathogenic parasitic eggs. Based on the data provided by the sanitary and epidemiological station, these deposits were widely used as material for land reclamation in Pińczów. The use of sludge as organic and mineral fertilisers should be cautious due to the potential source of pathogenic microorganisms (Andres, 1999; Sahlström et al., 2004). This is evidenced by the registered outbreaks of epidemics caused by soil contamination in Poland and Europe after the use of sewage sludge (Kłapć and Cholewa, 2012). Nevertheless, the use of sewage sludge may be useful in the reclamation of 'sterile' areas and as a natural fertiliser to the soil, but after careful examination (Dumontet et al., 2011, Kłapeć

and Cholewa, 2012, Niazi et al., 2015). A further goal of the research was to determine the microbiological purity of the air near two wastewater treatment plants (in Stryków and Szczecin, in the Świętokrzyskie province) based on the total number of mesophilic and psychrophilic bacteria and *Staphylococcus* sp. It was shown that the total number of bacteria isolated from the air from each site was acceptable. There was no relationship between the number of microorganisms in the air and the distance from individual sewage treatment plants. What is more, the results indicated even more bacteria at a further distance from sewage treatment plants. It is known that the number of bacteria in the air may depend on the wind direction, temperature and intensity of operation in sewage treatment plants (Filipkowska et al., 2000, Niazi et al., 2015). The number of detected bacteria seems to be lower in comparison to the research conducted in other wastewater treatment plants (Cyprowski and Krajewski, 2003; Okoch et al., 2007, Breza-Boruta, 2010, Budzińska et al., 2011, Niazi et al., 2015). These differences may result from a different size of the facility and the amount of sewage entering the treatment plant. Budzińska et al. (2011) also identified a large number of *Pseudomonas fluorescens* in the studied sewage treatment plants. These bacteria live naturally in heavily polluted surface waters and wastewater (Salyers and Whitt, 2005). In our study, we analysed the presence of a total number of bacteria and mannitol-positive *Staphylococcus* spp.. These indicators seem to be more important epidemiologically due to the potential risk of pathogenic bacteria for humans. It is worth noting that sewage treatment plants can also affect the sanitary state of local rivers. In addition, the literature indicates that the spread of bacteria from wastewater treatment plants may contribute to spread of drug resistance genes among environmental microorganisms and natural intestinal commensals (Huang et al., 2012; Li et al. , 2015). In conclusion, our results and other research indicate that the use of sewage sludge for land reclamation is helpful in maintaining and restoring the ecological balance of minerals, which is an important aspect of economic protection and the environment. However, it should be remembered that wastewater treatment plants as well as their sewage sludge may have an impact on air and soil pollution, creating a danger for the public health. It seems that microbiological monitoring of the environment should be carried out more frequently and in a wider scope.

Further cooperation with the sanitary and epidemiological stations also provided important observations on the microbial contamination of food products. The results of available microbiological tests of food products in 2008-2011 were published in **Microbiological contamination of food, Adamus-Bialek W.*, Wawszczak M., Ecol Chem Eng A, 2015, 22(4):509-516.** Various types of food products were analysed (meat, dairy products, cereal products, fish, vegetables, fruits, water, non-alcoholic drinks, vegetable fats, herbs, coffee, tea, cocoa, foodstuffs for specific nutritional uses and dietary supplements) of national or imported origin. The presence of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., *Yersinia enterocolitica* was determined. The *Escherichia coli* and *Yersinia enterocolitica* were detected the least often, and *Salmonella* spp. was detected most often, which is also responsible for the largest number of food poisoning in Poland. *Salmonella* spp. was detected mainly in domestic products such as poultry, eggs and egg products, cereal and cereal products,

coffee, cocoa, tea, convenience products and dietary supplements. In addition, all confectionery products were positive for the presence of *Salmonella* spp. In the subsequent years an increase of the frequency of these bacteria was observed. The presence of *Listeria monocytogenes* was most often detected in confectionery, ready meals, milk and dairy products, most rarely in fruits and vegetables. It should be emphasised that all analysed samples contained at least one of the tested bacterial species. Typical environments of particular bacterial species are known (Muskalska and Szymczak, 2015; Padungtod, 2006; Williams et al., 2015) but the widespread presence of *E. coli*, *Y. enterocolitica*, *L. monocytogenes* and especially all *Salmonella* spp. detected in food products is quite surprising. *E. coli* was even detected in bottled mineral water and other beverages. For example, detected bacteria in tap water disqualify it as fit for consumption and use in households. It should be remembered that food contamination may also increase the incidence of food poisoning as well as other diseases caused by these pathogenic bacteria. It is important to follow the hygiene rules during the production, preparation and consumption of food products. It is worth to emphasise, however, that the presence of pathogenic bacteria in food does not always have to be associated with the occurrence of the disease after ingestion. The infectious dose of the microorganism is also important. In addition, every organism has defence mechanisms that prevent bacterial infections. The ability to defend is an individual feature and determines the occurrence of the disease (Ferlazzo et al., 2002, Wardemann et al., 2002, Dempsey et al., 2003, Kopp and Medzhitov, 2003, Sochocka and Błach-Olszewska, 2005). However, it is important for the public to be aware of the threats and methods of preventing the spread of infectious diseases. These issues should be more widely publicised. Summing up the sanitary analysis of different environments, it should be emphasised that they are fully in line with the priority actions in the field of health promotion of the National Health Program aimed at reducing exposure to harmful factors in the living and working environment and improving the sanitary state of the country.

The scope of my research also concerned the analysis of factors influencing bacterial pathogenicity. Besides the pathogenicity of *Escherichia coli*, which was my scientific achievement, I participated also in studies on the pathogenicity of *Pseudomonas aeruginosa*, thanks to the cooperation with dr hab. Michał Arabski, who was in the team of professor Wiesław Kaca. The aim of the study was to determine the effect of selected heavy metal complexes on the production of pyocyanin and pyoverdine and the biofilm formation of clinical strains of *Pseudomonas aeruginosa*. The obtained results were published in **The effects of nickel(II) complexes with imidazole derivatives on pyocyanin and pyoverdine production by Pseudomonas aeruginosa strains isolated from cystic fibrosis, Gałczyńska K., Kurdziel K., Adamus-Białek W., Wąsik S., Szary K., Drabik M., Węgierek-Ciuk A., Lankoff A., Arabski M., Acta Biochimica Polonica, 2015, 62(4):739-745.** The infections caused by *Pseudomonas aeruginosa* are persistent and difficult to treat, especially for patients suffering from cystic fibrosis. Cystic fibrosis is an inherited disease characterised by chronic inflammation of the airways leading to respiratory failure. It causes the accumulation of mucus in the lungs, which increases the patient's compliance with bacterial infections that lead to progressive lung

damage and emphysema. The accumulated mucus in the lungs is an excellent environment for the formation of bacterial biofilm, which handicamps the bactericidal effect of the antibiotic and causes serious therapeutic problems (Stewart and Costerton, 2003; Manago et al., 2015). *P. aeruginosa* is a typical aetiologic agent of respiratory infections in patients with cystic fibrosis. Its specific properties include the ability to produce pyocyanin and pyoverdine (Kolpen et al., 2014, Nguyen et al., 2014, Muller and Merrett, 2015). Pyocyanin is a colourful compound with the ability to receive and donate electrons. It is able to damage cilia of respiratory epithelium, increases IL-8 secretion by epithelial cells, induces apoptosis and inhibits T-cell proliferation (Gloyne et al., 2011). Pyoverdine is a siderophore, binds and transports iron ions to a bacterial cell. It has also been proven to play a key role in biofilm formation, regardless of the presence of iron (Meyer et al., 1996; Jimenez et al., 2010). The aim of the research was to analyse the impact $NiCl_2$, newly synthesised two nickel complexes (II) ($[Ni(1\text{-}allim)_6](NO_3)_2$, $[Ni(iaa)_2(H_2O)_2]\times H_2O$) and their ligands in concentrations 7 – 500 μM on the production of pyocyanin and pyoverdine by 23 clinical strains of *P. aeruginosa* isolated from patients with cystic fibrosis. The concentrations of compounds were non-toxic to eukaryotic cells of the A549 line. There was no reduction in the production of pyocyanin or pyoverdine in the case of any studied strain upon treatment with $[Ni(iaa)_2(H_2O)_2]\times H_2O$. $[Ni(1\text{-}allim)_6](NO_3)_2$, $NiCl_2$ and their ligands (imidazole-4-acetate anion and 1-allylimidazoles) reduced the production of pyoverdine among 40% of the tested *P. aeruginosa* strains. Similar studies by other authors present the bactericidal effect of compounds with similar imidazole derivatives used at much higher concentrations (De-sai et al., 2013; Vijesh et al., 2011). The antibacterial properties of imidazole derivatives have been determined, for example, for 1-alkylimidazole (Khabnadideh et al., 2003), 2- (phenyl) -1H-imidazole and others (Sharma et al., 2009; Khalafi-Nezhad et al., 2005 Khan et al., 2008). An imidazole 4-acetate anion and 1-allylimidazole showed good diffusion properties by the mature biofilm of *P. aeruginosa* PAO1, measured by laser interferometry and confocal microscopy. The value of diffusion coefficient of imidazole-4-acetate in the biofilm was twice higher in comparison to 1-allylimidazole. These values are similar to the ciprofloxacin diffusion rate (Arabski et al., 2013) used in the treatment of infection in cystic fibrosis patients. Therefore, they may be considered for chemical synthesis with other metals, e.g. which are iron antagonistic as cobalt or gallium. Obtained results are an example of research on alternative solutions for antibiotics or as factors strengthening the antibiotic therapy of drug-resistant infections.

Considering my current achievements and scientific interests, I decided to transfer my employment to the Faculty of Medicine and Health Sciences of the same university. The scope of my previous research included the area of clinical and sanitary microbiology, so I decided that work at the Faculty of Medicine and Health Sciences will be more compatible with my scientific interests and will enrich my further scientific development towards healthcare and analysis of health problems in people. After a year's work at the Department of Microbiology at the Institute of Medical Sciences, I became the head of the Laboratory of Genetics at the Department of Surgery and Surgical Nursing under the supervision of prof. Stanisław Głuszek. At the same time, I took care of the clinical material (blood,

tissue) collected from over 1000 patients and I was involved in the research on genetic determinants of pancreatic diseases and metabolic syndrome. Working in a new team opened up further opportunities for scientific cooperation, which resulted in the participation in the publication **Omentin rs2274907 gene polymorphism and the risk of metabolic syndrome: a preliminary report, Suliga E., Kozieł D., Cieśla E., Rębak D., Wawszczak M., Adamus-Białek W., Naszydłowska E., Piechowska A., Głuszek S., Medical Studies, 2018, 34(4):267–275.** The research included 108 people diagnosed with the metabolic syndrome (MetS) and from 111 people representing the control group. Each person has been subjected to clinical examination and rP2274907 polymorphism analysis of the omentin-1 gene were performed by PCR-RFLP. The work presents for the first time an analysis of the potential relationship between omentin gene rs2274907 polymorphism and the risk of MetS. Metabolic syndrome is the accumulation of risk factors such as: abdominal obesity, dyslipidaemia, abnormal blood glucose and elevated blood pressure (Alberti et al., 2009, Rębak et al., 2015). The literature indicates the correlation between the concentration of omentin and the occurrence of certain biochemical parameters or risk factors of MetS (Auguet et al., 2011, Herder et al., 2017), but the results are not clear. Probably because the metabolic syndrome is affected by many genetic and environmental factors and their interactions. The obtained results did not show any significant relationship between omentin polymorphism Val109Asp and MetS risk or its components. We observed only a tendency for the Val/Asp genotype to be more frequent in people with metabolic syndrome compared to the control group and the more frequent occurrence of the Asp/Asp genotype in the control group in comparison with people with metabolic syndrome. Research on the expression and polymorphism of omentin genes in relation to metabolic risk factors are one of the few. Based on our analysis, only the potential indirect influence of genetic factors and their significance for other diseases such as obesity, type 2 diabetes, non-alcoholic steatohepatic disease (NAFLD) and the coronary artery for which the metabolic syndrome is a risk factor can be concluded. It is necessary to conduct further research on a larger population. Undoubtedly, the search for genetic links with metabolic diseases is a very promising direction in the prevention of civilization diseases.

5.2. Bibliometric evaluation

I. Original full-text scientific articles (excluding abstracts and conference abstracts, articles in journal supplements, letters to the editor and the author's share mentioned in the Appendix as a participant of multicentre studies, reviews):

A. In journals with Impact factors:

Year	Journal	Number of articles	IF	MNiSW points
2009	Journal of Clinical Microbiology	1	4.162	24
2011	Journal of Microbiological Methods	1	2.086	25
2013	Acta Biochimica Polonica	2	2.778	30
2013	Molecular Biology Reports	1	1.958	20
2015	Acta Biochimica Polonica	2	2.374	30
2017	Molecular Biology Reports	1	1.889	15
2018	Molecular Biology Reports	1	1.889	15
2019	Microbial Pathogenesis	1	2.332	20
2019	Virulence	1	3.947	35
In total:		**11**	**23.415**	**214**

B. In journals without Impact Factor:

Year	Journal	Number of aritcles	MNiSW points
2013	Journal of Spectroscopy	1	0
2014	Rocznik Świętokrzyski seria B – nauki przyrodnicze	2	2
2015	Ecological Chemistry and Engineering. A	1	11
2015	Proceeding of Ecopole	1	9
2018	Studia Medyczne/Medical Studies	1	10
2019	Studia Medyczne/Medical Studies	1	10
In total:		**7**	**42**

Case reports:

A. In journals with Impact factors: 0
B. In journals without Impact Factor: 0

Review articles:

A. In journals with Impact factors: 0
B. In journals without Impact Factor: 0

II. Book publications (authorship or co-authorship):

A. In a foreign language: number: 0
B. In Polish: number: 0

III. Chapters in compact publications:

A. In a foreign language: number: 0
B. In Polish: number: 0

IV. Chapter in the post-conference publishing: number: 1 MNiSW: 5

V. Editor-in-Chief of the journal with the range of:

A. International: number:0
B. National: number: 0

VI. Editor-in-chief of a multi-author book publication:

A. In a foreign language: number: 0
B. In Polish: number: 0

Additional information:

VII. Number of conference abstracts: 28
VIII. Popular science and other works: 0
IX. Number of publications in magazine supplements: 0
X. Number of publications with the author's participation in multicentre studies: 0

Total numer of articles:	**40**
Total numer of Impact Factor points:	**23.415**
Total numer of MNiSW points:	**261**
Number of citations (Web of Science Core Collection):	
Total number of citations:	**69**
The number of citations without self-citations :	**55**
Hirsch index:	**4**

5.3. International and national project management and participation in such projects

During my scientific work I participated in five scientific projects. One of them was implemented as a supervisor's grant during my doctoral thesis. After the Ph.D. studies I participated in other projects, being a project manager or co-investigator. The most important for my scientific achievements were: supervisor's grant N404 097 032/3354 (health sciences panel) financed by the Ministry of Science and Higher Education and Sonata project no. 2011/01/D/NZ7/00107 (health sciences panel) financed by the National Science Centre.

In the project N404 097 032/3354 'The development of a molecular method for diagnosis and differentiation of uropathogenic *Escherichia coli* strains based on the multiplex-PCR and TRS-PCR techniques' I was the main co-investigator. The obtained results were the basis for the doctoral dissertation. The project was financed in the amount of PLN 54 600. The results of the study were presented at 4 national and international conferences and published in the work of Adamus-Bialek W.*, Wojtasik A., Majchrzak M., Sosnowski M., Parniewski P., (CGG)4-based PCR as a Novel Tool for Uropathogenic Escherichia coli Discrimination: Comparison with ERIC-PCR, Journal of Clinical Microbiology, 2009, 47 (12): 3937-44 (IF: 4,162, MNiSzW 35).

In project 2011/01/D/NZ7/00107 'Epidemiological analysis of uropathogenic *Escherichia coli* strains' I was the leader and the main co-investigator. The project was financed in the amount of PLN 196,115.00. The obtained results were presented at numerous national and international conferences and published in 8 original articles in JCR journals with a total Impact Factor number of 15,602. Most of the mentioned works were the basis of my scientific achievement in habilitation proceedings:

- Adamus-Bialek W*, Zajac E, Parniewski P, Kaca W, Comparison of antibiotic resistance patterns in collections of Escherichia coli and Proteus mirabilis uropathogenic strains, Mol Biol Rep, 2013, 40(4):3429-35 (IF: 1,958)
- Lechowicz L., Adamus-Bialek W., Kaca W.; Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy and Artificial Neural Networks Applied to Differentiate Escherichia coli papG+/papG- strains., Journal of Spectroscopy, 2013, (2013), Article ID 538686, 3 pages, http://dx.doi.org/10.1155/2013/538686
- Adamus-Białek W*, Kubiak A, Czerwonka G, Analysis of uropathogenic Escherichia coli biofilm formation under different growth conditions, Acta Biochim Pol, 2015, 62(4): 765-71 (IF: 1,187)
- Czerwonka G, Guzy A, Kałuża K, Grosicka M, Dańczuk M, Lechowicz Ł, Gmiter D, Kowalczyk P, Kaca W, The role of Proteus mirabilis cell wall features in biofilm formation, Arch Microbiol. 2016, 198(9):877-84. doi: 10.1007/s00203-016-1249-x. (IF 1,607)
- Adamus-Bialek W*, Lechowicz Ł., Kubiak-Szeligowska AB, Wawszczak M, Kamińska E, Chrapek M, A new look at the drug-resistance investigation of uropathogenic E. coli strains; Mol Biol Rep, 2017, 44(1):191-202 (IF: 1,889)
- Adamus-Białek W*, Baraniak A, Wawszczak M, Głuszek S, Gad B, Wróbel K, Bator P, Majchrzak M, Parniewski P, The genetic background of antibiotic resistance among clinical uropathogenic Escherichia coli strains, Mol Biol Rep, 2018, pp 1-11 (IF: 1,889)

- Adamus-Białek W*, Vollmerhausen TL, Janik K, Hydrogen peroxide stimulates uropathogenic Escherichia coli strains to cellulose production, Microb Pathog, 2019, 126:287-291 (IF 2,332)
- Adamus-Białek W*, Wawszczak M, Arabski M, Majchrzak M, Gulba M, Jarych D, Parniewski P, Głuszek S, Ciprofloxacin, amoxicillin, and aminoglycosides stimulate genetic and phenotypic changes in uropathogenic Escherichia coli strains, Virulence, 2019, 10(1): 260-276 (IF: 3,947)

The list of projects in which I participated was listed in the table below:

Project title	**Project number,** implementation period, name of the institution awarding funds	The role in the project
The development of a molecular method for diagnosis and differentiation of uropathogenic *Escherichia coli* strains based on the multiplex-PCR and TRS-PCR techniques	**N404 097 032/3354**, 2007 – 2008, Ministry of Science and Higher Education	main investigator - PhD grant
Modification of ureolytic activity and biofilm formation in the environment by *Proteus mirabilis* strains in the presence of signalling substances	**NN 304044639**, 2011 – 2013, Ministry of Science and Higher Education	co-investigator
Epidemiological analysis of uropathogenic *Escherichia coli* strains	**2011/01/D/NZ7/00107**, 2011 – 2015, National Science Centre	Project Leader
The importance of bacterial biofilm in the environment. An attempt to determine the ability of bacteria to biofilm formation by colourimetric method	**496/W/10**, 2009 – 2010, Project of statutory research of UJK - Young Staff	Project Leader
Analysis of ability of *Escherichia coli* strains, isolated from different environments, to biofilm formation by genetic and phenotypic methods	**046/R/11**, 2011 – 2013, Project of statutory research of UJK - Young Staff	Project Leader

6. Didactic and popularizing achievements as well as information about the international cooperation of the postdoctoral student

6.1. Participation in European programs and other international and national programs

I took part in the following didactic and academic programs:

1. 'PROGRES - Development program: Economy-Education-Success' (POKL.04.01.01-00-077/10)

The program was implemented by the Jan Kochanowski University in Kielce as part of the Human Capital Operational Program, Priority IV Higher education and science, sub-operation 4.1.1 Strengthening the didactic potential of the university. From August to December 2012 I completed a scientific and didactic internship at the Karolinska Institute: Department of Microbiology, Tumour and Cell Biology (MTC) Stockholm in Sweden.

2. Grant PROGRES II

The program was implemented by the Jan Kochanowski University in Kielce. As a tutor of students from 23 to 28 September 2013 I took part in a scientific trip of second-year biotechnology students covered by the program. During the trip we were acquainted with the infrastructure and scientific and didactic activity of the Department of Chemistry, Department of Applied Genetics and Cell Biology and Department of Biotechnology University of Natural Resources and Life Sciences in Vienna (Austria) and 2nd Faculty of Medicine, Charles University and University Hospital Motol in Prague (Czech Republic).

3. 'PROGRES - Development program: Economy-Education-Success' (POKL.04.01.01-00-077/10)

The program was implemented by the Jan Kochanowski University in Kielce as part of the Human Capital Operational Program, Priority IV Higher education and science, sub-operation 4.1.1 Strengthening the didactic potential of the university. In May - July 2014, I completed a scientific and didactic internship at the School of Biological Sciences, University of East Anglia, Norwich in England.

4. ERASMUS+

Since 2017 I have also been a coordinator of the Erasmus + program of the Institute of Medical Sciences of the Faculty of Medicine and Health Sciences of UJK in Kielce.

6.2. Active participation in international and national scientific conferences

I am the co-author of 28 conference reports, on which the research results were presented. I took active part in 20 national, international or foreign conferences. I presented a lecture 'Genetic studies in pancreatic cancer' on the last conference 'VI Symposium Progress of Surgery'.

The list of conferences and presented studies:

1. Adamus-Białek W., Parniewski P., Kaca W., 2005, Molecular diagnostics of the uropathogenic strains of *Escherichia coli*, a work published entirely in the conference materials of the **8th Conference: 'Molecular biology in the diagnosis of infectious diseases and biotechnology'** SGGW, Warsaw, Poland
2. Adamus-Białek W., Sosnowski M., Parniewski P., 2007, TRS-PCR-based methodology for diagnostics, differentiation and epidemiology of UPEC strains, **13th European Congress on Biotechnology**, Barcelona, Spain
3. Parniewski P., Wojtasik A., Majchrzak M., Adamus-Białek W., Augustynowicz-Kopec E., Zwolska Z., Dziadek J., 2008, New method of genetic identification and differentiation of Mycobacterium strains, **XXVI Conference of Polish Society of Microbiologists**, Szczecin, Poland
4. Adamus-Bialek W., Wojtasik A., Majchrzak M., Parniewski P., 2009, TRS-PCR as a novel tool for uropathogenic Escherichia coli strains discrimination, **III National Conference BioMillenium 'Molecular Biotechnology'**, Gdansk University of Technology, Gdansk, Poland, poster
5. Majchrzak M., Wojtasik A., Adamus-Białek W., Parniewski P., 2010, Trinucleotide repeat sequences in epidemiological investigations, **The First International Conference of Biological Sciences**, Cairo, Egypt
6. Wojtasik A., Majchrzak M., Adamus-Białek W., Augustynowicz-Kopec E., Zwolska Z., Dziadek J., Parniewski P., 2010, Trinucleotide Repeat Sequences-based PCR as a Potential Approach for Genotyping Mycobacterium gordonae Strains, **Nucleic Acids Conference**, Cancun/Puerto Morelos, Mexico
7. Adamus-Białek W., Zawadzki K., Kwinkowski M., Parniewski P, Kaca W., 2010, Resistance patterns of clinical isolates of uropathogenic Proteus mirabilis and E. coli and their β-lactamases diversity, **II workshop MIKROBIOT 'Health and Environment Microbiology'**, University of Lodz, Poland
8. Glenska J., Adamus-Białek W., Kwinkowski M., Kolesinska B., Kaminski Z., Kaca W., 2011, The synthetic peptide library as tools for human antibody differentiation, **IV Congress of Polish Biotechnology and IV Eurobiotech 2011 "Four colours of biotechnology' Central European Congress of Life Sciences**, Krakow, Poland;

9. Lechowicz Ł., Adamus-Białek W., Kaca W., 2012, Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy as a method for differentiation of clinical Escherichia coli strains, **VII Conference 'Young scientists towards the challenges of modern technology'**, Warsaw, Poland,
10. Adamus-Białek W., Zajac E., Parniewski P., Kaca W., 2012, Analysis of drug resistance of uropathogenic Escherichia coli and Proteus mirabilis strains, **XXVII CONGRESS Polish Society of Microbiologists: 'Microbes without borders'**, Lublin, Poland,
11. Karwacka K., Palacz T., Bąk Ł., Adamus-Białek W., 2013, The microflora of selected water reservoirs in Świętokrzyskie Voivodship. **3rd Workshop on Microbiology in Health and Environmental Protection MIKROBIOT**, Łódź, Poland,
12. Lechowicz Ł., Urbaniak M., Adamus-Białek W., Kaca W., 2013, The use of IR spectrometry and Artificial Neural Networks to detect the susceptibility of E. coli to cephalothin, 3rd Workshop on Microbiology in Health and Environmental Protection MIKROBIOT, Lódź, Poland,
13. Wawszczak M., Kamińska E., Adamus-Białek W., 2014, Evaluation of susceptibility of uropathogenic *Escherichia coli* strains, **III. Student Conference of Molecular Biology**; Łódź; Poland
14. Lechowicz Ł., Urbaniak M., Adamus-Białek W., Kaca W., 2014, Differentiation of ampicillin-resistant and ampicillin-sensitive Escherichia coli strains by using infrared spectroscopy and multilayer perceptron, **1st Congress of the Polish Biochemistry, Cell Biology, Biophysics and Bioinformatics BIO2014**; Warsaw; Poland,
15. Adamus-Białek W, Majchrzak M., Gad B., Mazur J., Kamińska E., Wawszczak M., Gniadkowski M., 2015, Genetic and phenotypic analysis of drug-resistance of uropathogenic Escherichia coli strains. **6th International Weigl Conference on Microbiology**, Gdańsk, Poland,
16. Adamus-Białek W., Derlatka D., Świercz M., Czerwonka G., 2015, Analysis of uropathogenic Escherichia coli forming biofilm upon different environment conditions. 6th International Weigl Conference on Microbiology, Gdańsk, Poland,
17. Gałczyńska K., Kurdziel K., Adamus-Białek W., Arabski M., 2015, The effect of nickel(II) chloride on biofilm formation by uropathogenic Escherichia coli strains. 6th International Weigl Conference on Microbiology, Gdańsk, Poland,
18. Gałczyńska K., Kurdziel K., Adamus-Białek W., Szary K., ArabskiM., 2015, The effects of nickel(II) complexes with imidazole derivatives on pyocyanin and pyoverdine production by Pseudomonas aeruginosa strains. 6th International Weigl Conference on Microbiology, Gdańsk, Poland,

19. Lechowicz Ł., Chrapek M., Urbaniak M., Adamus-Białek W., Malinowska-Gniewosz A., Kaca W., 2015, Analysis of variability of uropathogenic Escherichia coli strains based on their infrared spectra, 6th International Weigl Conference on Microbiology, Gdańsk, Poland,
20. Wawszczak M., Adamus-Białek W., 2015, Microbiological contamination of food, **Central European Conference ECOpole'15**, Jarnołtówek, Poland,
21. Adamus-Białek M., Wawszczak M., Świercz A., 2015, Impact of sewage treatment plant on local environment, Central European Conference ECOpole'15, Jarnołtówek, Poland,
22. Adamus-Białek W., Filipiak A., Gwizda E., Kaleta J., Mąkosa M., Kaca W., Gniadkowski M., 2016, Genetic and phenotypic analysis of virulence factors of uropathogenic *Proteus mirabilis* strains, **XXVIII Congress of the Polish Society of Microbiologists**, Bydgoszcz, Poland,
23. Adamus-Białek W., Wawszczak M., Głuszek S., Bucki R., Parniewski P., Bowater R., 2017, Factors that influence the genetic stability of CRISPR/cas systems in Escherichia coli, **"Microbiology in Health Care and Environmental Protection' - MIKROBIOT 2017,** Łódź, Poland
24. Adamus-Białek W., Wawszczak M., Mazur J., Parniewski P., 2017, Subinhibitory concentration of antibiotics changes the bacteria, Microbiology in Health Care and Environmental Protection' - MIKROBIOT 2017, Łódź, Poland
25. Gluszek S., Wawszczak M., Majchrzak M., Adamus-Białek W., Klusek J., Nawacki L., Kozieł D., 2018, The mutations of carboxypeptidase 1 predisposes to distinguishing patients with acute pancreatitis and pancreatic cancer, **50th EPC The jubilee meeting of the European Pancreatic Club, 10th International Symposium on Inherited Diseases of the Pancreas**, Berlin, Germany
26. Kozieł D., Matykiewicz J., Wawszczak M., Majchrzak M., Adamus-Bialek W., Gluszek S., 2018, Follow-up as analysis tool of the potential long-term effects of SPINK, CTRC mutations in Acute Pancreatitis Patients, 50th EPC The jubilee meeting of the European Pancreatic Club, 10th International Symposium on Inherited Diseases of the Pancreas, Berlin, Germany
27. Kujko A.A., Oracz G., Fjeld K., Wejnarska K., Wertheim-Tysarowska K., Kołodziejczyk E., Bal J., Adamus-Białek W., Kozieł D., Kowalik A., Głuszek S., Molven A., Rygiel A.M., 2018, Association between CEL-HYB1 allele and idiopathic/familial chronic pancreatitis in Polish pediatric patients, 50th EPC The jubilee meeting of the European Pancreatic Club, 10th International Symposium on Inherited Diseases of the Pancreas, Berlin, Germany
28. Gluszek S, Wawszczak M, Adamus-Bialek W, Majchrzak M, Klusek J, Kozieł D, 2018, Coexistence of CPA1, SPINK1, CFTR, CTRC, PRSS1 Gene Mutations in Acute Pancreatitis and Pancreatic Cancer, PANCREAS, 2018, **49th Annual Meeting of the American Pancreatic Association**, Miami Beach, Florida, USA
29. Adamus-Białek W., lecture 'Genetic studies in pancreatic cancer', VI Symposium Progress of Surgery, 2019, Kielce, Poland

6.3. Participation in editorial board and scientific councils of journals

In 2015 – 2017, I was a member of the editorial board of three journals:

1. Journal of Agricultural Science and Engineering. American Institute of Science – Public Science Framework, member of the scientific council - a reviewer, a member of the editorial board
2. Public Health and Preventive Medicine, American Institute of Science – Public Science Framework, member of the scientific council - a reviewer, a member of the editorial board
3. Clinical Medicine Journal, American Institute of Science – Public Science Framework, member of the scientific council - a reviewer, a member of the editorial board

6.4. Membership in international and national organizations and scientific societies

I am a co-founder and board member of the Kielce branch of Polish Society of Microbiologists:

- 2009 - 2012 – secretary of the Kielce branch of Polish Society of Microbiologists,
- 2012 - 2016 – the vice-chairman of the Kielce branch of Polish Society of Microbiologists,
- 2016 – currently – the chairman of the Kielce branch of Polish Society of Microbiologists.

6.5. Scientific care for students

In the years 2009 – 2017 I was the supervisor of 48 bachelor's theses in the field of the environmental protection or biotechnology of the Faculty of Mathematics and Natural Sciences at the Jan Kochanowski University in Kielce. All works, except one, included experimental analysis. I was also the supervisor of 7 master's thesis on the environmental protection or biotechnology of the Faculty of Mathematics and Natural Sciences at the Jan Kochanowski University in Kielce. All works included experimental analysis. The results obtained through the cooperation with the students constituted a partial contribution to the 7 scientific publications of my scientific achievements.

A detailed list of diploma theses (Name and surname of student, title of thesis, field of study, year):

The titles of final work of Bachelor's studies:

1. Przemysław Wieliński, Macroscopic analysis of bacterial cultures isolated from air pollution prepared in the Base Station of the Holy Cross, environmental protection, 2009
2. Monika Bębenek, Microscopic analysis of bacteria isolated from the dust of the air of the Base Station of the Holy Cross, environmental protection, 2009
3. Marta Okoń, Microbiological characteristics of air as a secondary environment for bacteria based on microbiological analysis of particulate pollutants isolated in the Base Station Holy Cross, environmental protection, 2009
4. Ewelina Tarłowska, Macroscopic analysis of fungi isolated from the air of the Holy Cross Mountains - Holy Cross, environmental protection, 2009

5. Katarzyna Ziętal, Microbiological analysis of air as an environment for bacteria based on microbiological analysis of dust pollutants, isolated in the Base Station of the Holy Cross, environmental protection, 2009
6. Anna Kabała, Study of the microbiological diversity of the air of the St. Cross of the Świętokrzyskie Mountains, protection of the environment, 2010
7. Marcin Zawada, The study of the total number of microorganisms present in the air isolated from the dust Base Station of the Holy Cross, environmental protection, 2010
8. Albert Adamczyk, City Park as refuge for a polluted urban environment based on the basic microbiological tests of air, environmental protection, 2010
9. Beata Pacek, The study of microbiological contamination of air near wastewater treatment plants, environmental protection, 2010
10. Szczepan Duralski, Microbiological analysis of air in places with special hygienic requirements on the example of a pharmacy, environmental protection, 2010
11. Mira Galus, Study of the impact of macro-environment on the microbiological diversity of air on the example of field and orchard, environmental protection, 2010
12. Justyna Kasperek, Study of the impact of macro-environment on the microbiological diversity of air on the example of deciduous forest and boron, environmental protection, 2010
13. Iwona Wojtasik, Microbiological analysis of indoor air on the example of cinema, environmental protection, 2010
14. Dorota Ziglińska, Microbiological analysis of activated sludge using selected molecular techniques, ochrona środowiska, 2010
15. Monika Żurawska, Determination of microbiological diversity of the Vistula River in the coastal zone, in Zawichost, environmental protection, 2010
16. Maciej Kusztal, Microbiological analysis of the Końskie water reservoir, environmental protection, 2011
17. Michał Michałek, Microbiological characteristics of air in Kielce, environmental protection, 2011
18. Joanna Niedziela-Wąsacz, Microbiological analysis of the coastal zone of surface water in Pińczów, on the example of selected research stands, environmental protection, 2011
19. Ewelina Sokołowska, Epidemiology of food product contamination in the Świętokrzyskie Province, environmental protection, 2011
20. Bożena Taborska, Analysis of microbiological purity of the stream, constituting the inflow of the Czarna Staszowska River in the Świętokrzyskie Province, environmental protection, 2011
21. Klaudia Tamborska, Environmental bacteria as a source of readily available biological weapons based on the analysis of available literature, environmental protection, 2011
22. Katarzyna Wąsowska, Microbiological analysis of the coastal zone of the Bernatka River, in Skarżysko-Kamienna, environmental protection, 2011
23. Edyta Włodarczyk, Influence of earthworm *Eisenia fetida* on microflora of bottom sediment at the Białogon housing estate in Kielce, environmental protection, 2011
24. Remigiusz Ziewiecki, Characteristics of the bacterial microflora of the coastal zone of the water reservoir, in the Wietrznia nature reserve in Kielce, environmental protection, 2011
25. Marzena Żydek, Microbiological analysis of bottom sediment at the Białogon housing estate in Kielce, environmental protection, 2011
26. Karolina Głuszek, The study of the impact of sewage treatment plants on the microbiological purity of air in the Szczecno, Świętokrzyskie province, environmental protection, 2012
27. Artur Stefański, The use of bacterial preparations in the cultivation of plants in the province Świętokrzyski, environmental protection, 2012
28. Ewelina Kamińska, Microbiological analysis of bathing areas in the Piotrków Trybunalski in 2008-2010, environmental protection, 2012
29. Klaudia Kulig, Occurrence and diagnosis of *Enterococcus faecalis* in surface water intakes on the Nida River, in 2006-2011, environmental protection, 2012

30. Paulina Kowalska, Occurrence of *Escherichia coli* in surface water in the Nida River, Świętokrzyskie Province, in the years 2005-2011, environmental protection, 2012
31. Ewelina Bech, Sewage sludge management at the sewage treatment plant in Pińczów, environmental protection, 2012
32. Martyna Drabik, Sanitary analysis of sewage sludge on the example of selected wastewater treatment plants in the Świętokrzyskie Province, environmental protection, 2012
33. Wioleta Nowaczek, The occurrence of microbiological indicator in food products on the example of the *Listeria monocytogenes* strains in Sandomierz, in the years 2008-2010, environmental protection, 2012
34. Diana Berlińska, Occurrence of environmental microorganisms causing contamination of food products, on the example of sample from Busko Zdrój in 2009 and 2011, environmental protection, 2013
35. Karolina Karwacka, Sanitary analysis of bottom sediments of selected water reservoirs of the Świętokrzyskie Province, Biotechnology, 2013
36. Tomasz Palacz, Microbiological sanitary analysis of selected water reservoirs of the Świętokrzyskie Province, biotechnology, 2013
37. Ewelina Kamińska, Evaluation of drug resistance of uropatogenic *Escherichia coli* strains to selected antibiotics, biotechnology, 2014
38. Krystsina Huleyeva, Identification of *sdiA* and *rcsA* genes determining the ability to biofilm formation in the uropathogenic *Escherichia coli* strains, biotechnology, 2014
39. Monika Wawszczak, Identification of extended-spectrum beta-lactamase producing strains in the collection of uropathogenic *Escherichia coli* strains, biotechnology, 2014
40. Sylwia Chrzanowska, Characteristics of *Escherichia coli* isolates based on sensitivity to selected antibiotics, environmental protection, 2015
41. Justyna Magdalena Mazur, Identification by PCR of drug resistance genes for beta-lactam antibiotics in the collection of uropathogenic *E. coli* strains, biotechnology, 2015
42. Beata Gad, Identification of drug resistance genes for selected antibiotics in the collection of uropathogenic *Escherichia coli* strains, biotechnology, 2015
43. Joanna Kaleta, The study of the ability to biofilm formation among *Proteus mirabilis* strains collection, environmental protection, 2016
44. Ewa Gwizda, The attempt to correlate the swarming growth with biofilm formation among *Proteus mirabilis* strains, biotechnology, 2016
45. Aneta Filipiak, The attempt to correlate the swarming growth with the ureolytic properties of *Proteus mirabilis*, biotechnology, 2016
46. Martyna Gulba, The influence of selected antibiotics on the intensity of biofilm formation of *Escherichia coli* strains, biotechnology, 2016
47. Paulina Bator, Analysis of gene sequences of topoisomerases of uropathogenic *Escherichia coli* by bioinformatic methods, biotechnology, 2017
48. Klaudia Wróbel, Comparison of gyrase gene sequences in uropathogenic *E. coli* strains by bioinformatic methods, biotechnology, 2017

The titles of final work of M.Sc. studies:

1. Joanna Niedziela-Wąsacz, Molecular analysis in correlation to phenotypic methods for the differentiation of environmental microorganisms, environmental protection, 2013
2. Katarzyna Wąsowska, Differentiation of microorganisms isolated from soils with phenotypic and genotypic methods, environmental protection, 2013
3. Anna Szafraniec, The influence of nickel on the growth and biofilm formation among *Proteus mirabilis* strains collection, environmental protection, 2015
4. Anna Szafraniec, Effect of nickel chloride on the growth and biofilm formation among *Escherichia coli* strains, biotechnology, 2016

5. Monika Wawszczak, The effect of subinhibitory concentration of antibiotic on changes in drug resistance of the uropathogenic *Escherichia coli* strains, biotechnology, 2016
6. Marzena Mąkosa, The study of selected virulence factors in the collection of uropathogenic clinical strains of *Proteus mirabilis*, biotechnology, 2017
7. Justyna Magdalena Mazur, Comparative analysis of mutants and wild type strains of *Escherichia coli* using the PCR method, biotechnology, 2017

Starting work at the Independent Environmental Protection and Modelling at the Jan Kochanowski University in 2008, I developed a syllabus and adapted the laboratory to didactic classes in the field of environmental protection in the subject of 'Genetics and Genetic Engineering', 'Microbiology', 'Microbiology of waters', 'Biochemical and biophysical analysis in contaminated systems' I also prepared a syllabus in the subject of 'Genetically modified organisms' in the field of biotechnology. Classes included laboratory, exercises and lectures.

While working at the Institute of Medical Sciences, I took part in the adaptation of laboratories and the development of didactic classes in the subject 'Microbiology', 'Parasitology', 'Genetics', 'Genetically modified food' in the field of medicine. I prepared and provided equivalent didactic classes (with the exception of microbiology) in the field of English-language medicine.

I am the coordinator of the Erasmus+ program at the Institute of Medical Sciences, The Faculty of Medicine and Health Sciences, Jan Kochanowski University.

6.6. Scientific care for PhD students as a scientific supervisor or auxiliary promoter

I am the auxiliary promoter of two doctoral theses:

• Andrzej Szczepanek, M.Sc., doctoral dissertation 'Factors determining the occurrence of *Legionella* bacteria in hospitals and social care homes in the Świętokrzyskie Voivodeship', an open doctoral dissertation in the field of health sciences, at the Faculty of Medicine and Health Sciences, UJK, supervisor dr hab. Grażyna Nowak- Starz, prof. UJK

• Monika Wawszczak, M.Sc., doctoral dissertation 'The role of carboxypeptidase in the mechanism of formation of acute pancreatitis', an open doctoral dissertation in the field of health sciences, at the Faculty of Medicine and Health Sciences, UJK, supervisor of prof. dr hab. n. med. Stanisław Głuszek

6.7. Scientific internships in foreign and national scientific or academic centres

Date, place	Purpose of stay and effects
VIII – XII. 2012 Karolinska Institute: Department of Microbiology, Tumour and Cell Biology (MTC), Stockholm, Sweden	Acquiring the skills of new microbiology and molecular biology techniques, participation in research: phenotypic and genetic analysis of uropatogenic *Escherichia coli* strains, the influence of oxygen free radicals on growth and biofilm of *E. coli*. The result is the establishment of scientific cooperation and publication Adamus-Białek W*, Vollmerhausen TL, Janik K, Hydrogen peroxide stimulates uropathogenic Escherichia coli strains to cellulose production, 2018, Microb Pathog, 126:287-291 (IF 2,332)
V – VII. 2014 School of Biological Sciences, University of East Anglia, Norwich, Anglia	Expanding the skills in molecular biology techniques. Study of CRISPR sequence stability in the genome of *Salmonella* spp. strains, preparation of a plasmid gene constructs containing repeated sequences TRS. The result is establishing scientific cooperation and obtaining preliminary results on the instability of CRISPR in the uropathogenic genomes of *Escherichia coli*. The promising results obtained and the established scientific cooperation are the basis for starting a new research direction and will allow for the preparation of the Sonata Bis project to the NCN.
I. 2017 Department of General Biology and Parasitology, Warsaw Medical University, Warsaw, Poland	Training experience – parasitological diagnostics. The internship served to broaden professional competences in the field of parasitology and didactic preparation for classes conducted in the newly opened medical field at the Faculty of Medicine and Health Sciences of UJK

6.8. Reviews of publications in the international and national journals

Since 2015 I have been invited to review manuscripts of original research and review works in international and foreign journals. In total, I reviewed 58 manuscripts in 31 journals, 11 of which have Impact Factor in the range from 0.5 - 13.7.

No.	Journal	Period	Number of reviewed manuscripts	Impact Factor (MNiSzW)
1.	Microbiology Research International	2015	1	0
2.	African Journal of Microbiology Research	2015 – 2016	3	0
3.	Clinical Medicine Journal	2015 – 2016	4	0
4.	Agricultural and Biological Sciences Journal	2015 – 2017	5	0
5.	Public Health Journal	2015	2	0
6.	Journal of Agricultural Science and Engineering	2015 – 2017	9	0
7.	International Journal of Preventive Medicine Research	2015	1	0
8.	American Journal of Clinical Neurology and Neurosurgery	2015	1	0
9.	Public Health and Preventive Medicine	2015 – 2016	2	0
10.	Acta Biochimica Polonica	2015	3	1,239 (15)
11.	Journal of Antimicrobial Chemotherapy	2015	1	5,217 (40)
12.	American Journal of Microbiology and Immunology	2016	2	0,91
13.	AASCIT Frontiers in Biomedical Sciences	2016 – 2017	2	0
14.	Studia Medyczne	2016 – 2018	3	(10)
15.	African Journal of Traditional, Complementary and Alternative Medicines	2017	1	0,553 (20)
16.	International Journal of Environmental Research and Public Health	2017	1	2,145 (30)
17.	ACS Nano	2017	1	13.709 (45)
18.	ACTA PHARMACEUTICA	2017	1	1,623 (20)
19.	European Journal of Biological Research	2018	1	0
20.	Genes	2018	1	3,191 (25)
21.	Microbial Cell Factories	2018	1	4,295 (40)
22.	Microbial Drug Resistance	2018	1	2,344 (25)
23.	Clinical Microbiology and Infectious Diseases	2018	2	0
24.	Biomolecules	2018	1	0
25.	JSM Biomedical Imaging Data Papers	2018	1	0
26.	Annals of Clinical and Medical Microbiology	2018	1	0
27.	Journal of Case Reports and Studies	2019	1	0

28.	African Health Sciences	2019	2	0,666 (20)
29.	Journal of Surgery and Surgical Research	2019	1	0
30.	Science Asia	2019	1	0
31.	Infection and Drug Resistance	2019	1	0

6.9. Scientific and research cooperation with other scientific centres

In the years 2009 – 2018, I established scientific cooperation with numerous scientific centres, which resulted in the acquisition of new scientific and laboratory experiments as well as joint publications, participation in conferences or scientific substantive support.

Scientific cooperation:

1. Laboratory of Molecular Genetics, Institute of Medical Biology of the Polish Academy of Sciences in Łódź, Paweł Parniewski Ph.D., prof. PAS
2. School of Biological Sciences, University of East Anglia, Norwich, prof Richard Bowater,
3. Department of Microbiology, Tumour and Cell Biology (MTC), Karolinska Institute, Stockholm, Sweden, prof. Annelie Brauner
4. Department of Geotechnics and Water Engineering, Kielce University of Technology, Kielce, Łukasz Bąk Ph.D.
5. National Medicines Institute, Warsaw, Poland, prof. Marek Gniadkowski, Anna Baraniak Ph.D.
6. Department of Microbiology, Institute of Biology, Faculty of Mathematics and Natural Sciences of the Jan Kochanowski University in Kielce, prof. Wiesław Kaca
7. Department of Biochemistry and Genetics, Institute of Biology, Faculty of Mathematics and Natural Sciences of the Jan Kochanowski University in Kielce, Michał Arabski Ph.D., prof. UJK,
8. Institute of Mathematics, Faculty of Mathematics and Natural Sciences of the Jan Kochanowski University in Kielce, Magdalena Chrapek Ph.D., Elżbieta Zając Ph.D.