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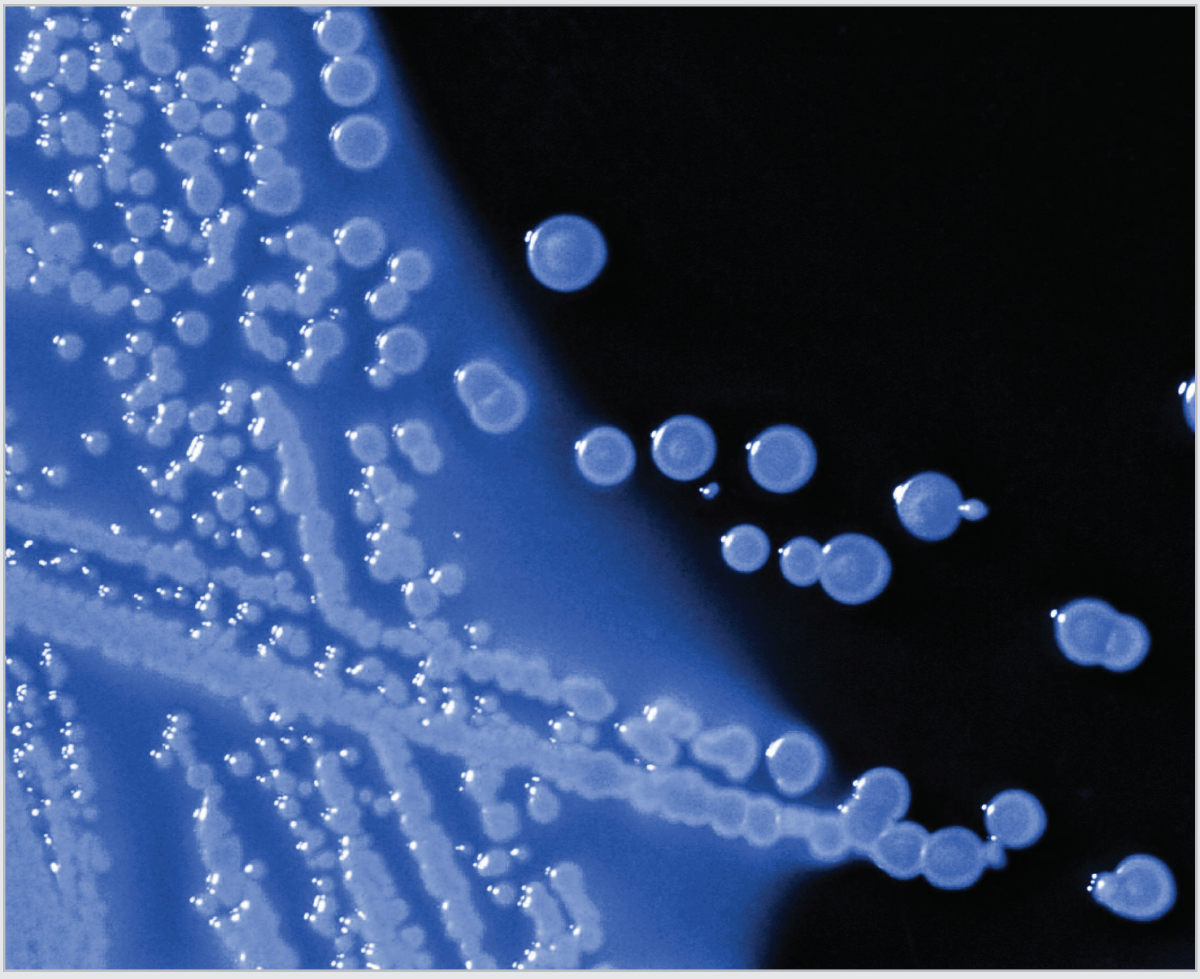
Acta Pharmaceutica Hungarica

Taxonomy and nomenclature of bacteria



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Taxonomy and nomenclature of bacteria with clinical and scientific importance: current concepts for pharmacists and pharmaceutical scientists

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Abstract
Taxonomy is the science of the classification of various living organisms consisting of three independent, but interrelated disciplines, namely classification, nomenclature and identification. With the advent of molecular biological methods and sequencing, a revolution is currently occurring with regards to the reporting of novel taxa and changes in the taxonomy of already described bacterial species. The applications of taxonomic changes can be broad ranging: they may impact the clinical care of patients, through variations in choosing the appropriate antimicrobial susceptibility testing standards or data interpretation, or even their clinical relevance and epidemiology. The aim of this paper was to aid healthcare professionals and pharmaceutical scientists to navigate through the 'maze' of bacterial taxonomy, and to aid in finding authentic information regarding the description of taxonomic changes and to present some examples of changes in bacterial taxonomy which have proven to be clinically significant.

Keywords: *bacteria, taxonomy, nomenclature, identification, molecular, microbiology, educational*

1. Introduction to (bacterial) taxonomy

Taxonomy (from the greek words *taxis*=arrangement or order, and *nemein*=to distribute or govern) is the science of the classification of various living organisms [1,2]. In case of bacteria, taxonomy consists of three independent, but interrelated disciplines, namely *classification*, *nomenclature* and *identification* (sometimes referred to as the 'trinity' of taxonomy) [2]. The most basic taxonomic group (i.e. unit) in bacterial taxonomy is the species, while groups of species are collected into genera (genus), which are then collected into families (*Familia*), families into orders (*Ordo*), orders into classes (*Classis*), classes into phyla (*Phylum*) and phyla into a domain (or *Kingdom*, the highest level), however, there are subgroups to these main classifications (see *Table I* and *II* for examples). Groups of bacteria at each rank or level have names with endings or suffixes characteristic to that rank or level (*Table I*) [1-3].

Nevertheless, taxonomic units under species may still be relevant (especially in the case of medically-relevant bacteria), because members among specific species can be distinguished on the basis of certain biological or genetic characteristics: these members may be classified in a sub-group of members, called *subspecies* [1-3]. An example for this is the differentiation of *Campylobacter* species*: C. fetus subsp. veneralis* is a causative agent of sexually transmitted diseases and miscarriage among cattle, while *C. fetus subsp. fetus* may cause intrauterine infection in humans [4]. Antigenic characteristics may be another possible way to distinguish subgroups under the threshold of species, called

Table I *Example of taxonomic classification for a common Gram-positive, Gram-negative and an atypical pathogen*

	Staphylococcus aureus	***Pseudomonas aeruginosa***	***Mycoplasma pneumoniae***
Kingdom	Bacteria	Bacteria	Bacteria
Phylum	Firmicutes	Proteobacteria	Tenericutes
Class	Bacilli	Gammaproteobacteria	Mollicutes
Order	*Bacillales*	*Pseudomonadales*	*Mycoplasmatales*
Family	*Staphylococcaceae*	*Pseudomonadaceae*	*Mycoplasmataceae*
Genus	*Staphylococcus*	*Pseudomonas*	*Mycoplasma*
Species	*S. aureus*	*P. aeruginosa*	*M. pneumoniae*

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serogroups or *serovariants* [5]. In case of gut bacteria or *Enterobacteriaceae* (especially important for *Salmonella* species and *Escherichia coli*), hundreds of different serovariants may be differentiated, based on the cell wall (O; somatic antigen, based on oligosaccharides), capsule (K, from the German *Kapsel* or *Bacterienkapsel*) and flagellar (H; from the German *Hauch* meaning "breath" or "mist") antigens [6,7]. In fact, this is the basis of the Kauffman–White classification, which was frequently used for routine clinical microbiology and public health purposes for serotyping [8]. Similarly, bacteria may be further characterized based on their disease-causing capacity (pathogenicity) into *pathotypes*, e.g., extraintestinal-pathogenic *E. coli* (ExPEC), enteropathogenic *E. coli* (EPEC), enterotoxin-producing *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and so on [9,10].

However, a lot has changed since the first description of taxonomy (Augustin Pyramus de Candolle, 1813), when the available methods for the characterization of bacterial, plant or animal species were very limited [11]. Nowadays, with the advent of molecular biological methods and sequencing, a revolution is currently occurring with regards to the reporting of novel taxa [12]. The description of new bacterial species was further facilitated by the newfound interest in the characterization of the human microbiome [13]. One of the most important milestones was the launch of the Human Microbiome Project (HMB; the first phase of which was launched in 2007), with the aim of characterize the human gut microbial flora in healthy (physiological) and disease states; the long-term aim of this project was to find causation between human pathologies (e.g., autoimmune disorders, obesity, diabetes, neuropsychiatric disorders, diseases affecting the cardiovascular system) and qualitative/quantitative changes in the microbiome [14-16]. Microbial culturomics (a technique allowing for the culturing of previously *unculturable* bacterial species by reproducing their natural habitats using complex methods, with the aid of matrix-assisted laser desorption-ionization time-of-flight mass spectrometry [MALDI-TOF MS] and whole-genome sequencing [WGS]) has also resulted in the description of a staggering number of novel taxa [17-19]. Sequencing technologies also had a significant role in the description of the prokaryotic genetic diversity.

Table II *Characteristics of the current bacterial classification and the number validly published names for each classification level [22]*

Taxonomical level		n
Kingdom	*Regnum*	1
Subkingdom	*Subregnum*	2
Infrakingdom	*Infraregnum*	2
Superdivision/Superphyla	*Superdivisio*	1
Subdivision/Subphyla	*Subdivisio*	9
Superclass	*Superclassis*	2
Class	*Classis*	106
Subclass	*Subclassis*	8
Order	*Ordo*	188
Suborder	*Subordo*	19
Family	*Familia*	399
Subfamily	*Subfamilia*	0
Tribe	*Tribus*	24
Subtribe	*Subtribus*	0
Genus	*Genus*	2854
Species	*Species*	15626
Subspecies	*Subspecies*	586

Between 1990 and 2000, there was on average 200 novel bacterial species described per year [20]. Owing to these recent developments, the number of validly published genera and species has increased by approximately 50% since 2004, reducing the percentage of known prokaryotes that have been implicated in animal or human clinical conditions from ~15% to ~10% [21]. Based on the records of the bacterio.net database, there are currently 19,717 *validly* published bacterial names and 383 so-called *candidate* names published (as of 20th of October, 2019) [22]. However, the database of EZBioCloud.net (a freely-accesible database on prokaryotic diversity) contains 81,189 taxa (out of which, 24.89% has been *validly* published and 0.51% are *candidate* names), 64,416 16S rRNA sequences (a highly conserved, evolutionally-constant region) and 146,704 qualified genomes (as of 9th of August, 2019) [23]. Nonetheless, there are reports estimating that the currently known/described microbiological diversity only represents around 1-5% of the global prokaryotic diversity [20-21].

Bacterial systematics is a field, which is frequently used synonymously with taxonomy, however, the scope of systematics is much broader, including data on bacterial morphology, physiology, molecular biology and biochemistry, metabolic products, pathogenic potential, ecological niches and epidemiology to characterize, arrange and

classify bacteria [24]. Systematics became more relevant after the widespread adoption of molecular biological methods, ever higher resolution characterization of bacterial species [25] (*Figure 1*).

Due to the rapid developments in bacterial taxonomy, both consisting of the description of novel taxa and reclassification of existing bacterial genera to other taxonomical units (e.g., the history of *S. maltophilia*: it first described as *Bacterium booker* (1943), later on, it was redesignated as *Pseudomonas maltophilia* (1958) and *Xanthomonas maltophilia* (1981); finally, in 1993, the genus *Stenotrophomonas* was proposed), it is very difficult, if not impossible for researchers, officials, public health microbiologists and healthcare professionals to keep in mind all the accepted or proposed changes [26,27]. However, the importance of correct taxonomy in scientific communication and the diagnostics and therapy of bacterial infections cannot be underestimated [3]. Even if they are not aware of all the changes and the newly introduced species, relevant persons should be able to quickly find them in medical literature, scientific publications or other sources (Web pages or blogs kept up by taxonomists).

There are various resources for pharmaceutical scientists and microbiologists to get informed regarding the recognition of novel bacterial species or describing proposed reclassification of an older species. The official publication prokaryotic taxonomy and source of data regarding these matters in the International Journal of Systematic and Evolutionary Microbiology (IJSEM); the main aim and role of this publication is to report the description of new taxa or the reclassification of existing species [28]. The rules associated with the proposal of new bacterial taxa were described in the *Bacteriological Code* (1990), which was updated though the publication of the Taxonomic Outline of the Bacteria and Archaea (TOBA; 2006) [29,30]. Additional amendments to these rules are generally published in IJSEM. The proposed new species and species names (*candidate*) are to be submitted to the Editorial Office of IJSEM for evaluation, with the suffix *nova* (genus *nova*, species *nova*). The new taxonomy and nomenclature can only be considered as official, if the Editorial Board of IJSEM and the International Committee on Systematics of Prokaryotes (ICSP) both approve the submission [21]. If approved, the proposed name receives the approved state (*valid name*), which is formalized by the certificate of approval awarded by the IJSEM and ICSP [21,28]. However, once these taxa are on the approved lists, they may still be subject to reclassification, based on the designation of synonyms or due to a transfer to another genus. The validation of a new taxa is finalized if designated type strains of the species are deposited into internationally-recognized culture collections (e.g., American Type Culture Collection [ATCC], Asian Bacterial Bank [ABB], Anaerobe Reference Laboratory, Helsinki Collection [AHN], Culture Collection of Switzerland [CCOS], Collection de l'Institut Pasteur [CIP], United Kingdom National Culture Collection [UKNCC]) at least in two separate countries [31].

The Antoine van Leeuwenhoek Journal of Microbiology has become the second main journal in this field in the recent years, reporting on >100 new candidate species per year, since 2014 [32]. In addition, several other journals with interests in microbiology/infectious diseases may be vehicles in reporting novel taxa, including but not limited to: Systematic and Applied Microbiology, Journal of Medical Microbiology, Current Microbiology, Clinical Microbiology and Infection, Diagnostic Microbiology and Infectious Disease, Anaerobe, Infection, Genetics and Evolution, Journal of Antimicrobial Chemotherapy, Emerging Microbes and Infections, New Microbes and New Infections, Microbiology and Immunology, Frontiers in Genetics, Frontiers in Microbiology, Archives of Microbiology, MicrobiologyOpen, Standards in Genomic Sci-

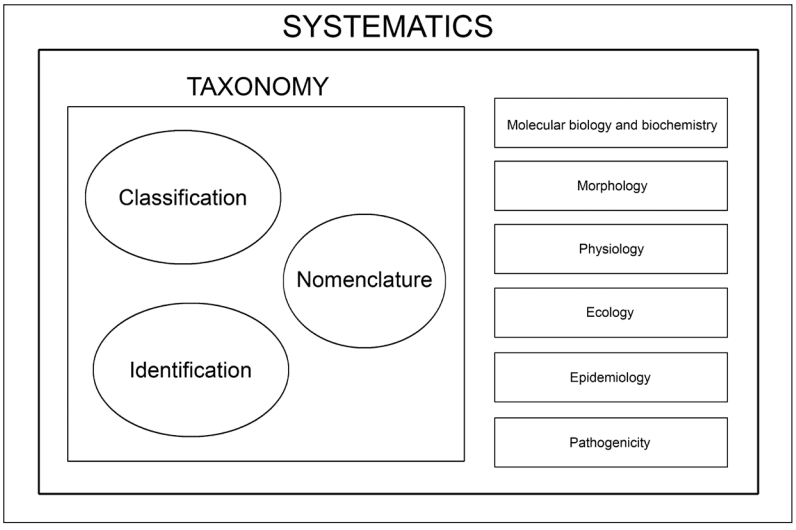

Figure 1 *Relationship of the field of taxonomy and bacterial systematics*

ences, Acta Pathologica Microbiologica et Immunologica Scandinavica (APMIS), Zentralblatt für Bakteriologie, Research in Microbiology [33]. Nevertheless, it is important to note that for the novel or revised taxa to be validly published (and the study was not submitted to IJSEM), the proposition must be included on an "approved" list in IJSEM. IJSEM publishes papers entitled "*List of new names and new combinations previously effectively, but not validly, published*" six-to-twelve times per year, which gives a good idea about the momentum of bacterial taxonomy [21,28,33]. The proposed taxa that have been previously described in other journals will be footnoted in IJSEM.

2. What is in a name: nomenclature in bacteriology

The discipline of *nomenclature* is mainly concerned with the assignment of names to taxonomic units or groups, on the basis of specific rules [34]. Before a name could be designated for any microorganism, one has to describe its biological characteristics (for its future identification), allowing for its classification in the subordinate system, as previously described [1-3]. The origins of nomenclature date back to 1753, when Carl Linnaeus published *Species Plantarum*, introducing the *binomial* nomenclature and the currently used classification hierarchy, based on greek-latin terms as the normal system of naming organisms [35]. Species Plantarum (later functioning as the International Code of Botanical Nomenclature [ICBN]) was first set of rules and recommendations of its kind. Because bacteria were once classified among plants, the nomenclature of these microorganisms was subject to the rules of the ICBN until the 1930s, when the bacteriological society has decided on the preparation of an independent code. The International Code of Bacteriological Nomenclature (or Bacteriological Code for short) was first approved in Copenhangen, 1947 [29,30]. The entire framework of naming bacteria is too complex to be described in its entirety, as the Bacteriological Code currently had more than 500 rules and regulations regarding name proposals for novel species names (which are periodically being updated; the updates are published in IJSEM) [36]. As the "intellectual capital" (i.e. available empirical and experimental data) available for the scientists submitting the candidate names for consideration constantly grows, so does the scientific accuracy of the bacterial names. The etymology (the study of the origin and history of words) of bacterial genus/species name is very diverse; here are several examples on the etymology of some bacterial genera:

- **Famous microbiologists (or scientists):** *Rothia* (Genevieve D. Roth), *Kingella* and *Elizabethkingia* (Elizabeth O. King), *Escherichia* (Theodor Escherich), *Pasterurella* (Louis Pasteur), *Gaffyka* (Georg Theodor A. Gaffky), *Burkholderia* (W.H. Burkholder), *Ehrlichia* (Paul Ehrlich), *Serratia* (Serafino Serrati, physicist)
- **Mythological names:** *Cronobacter* (Cronos, a titan), *Proteus* (Proteus, a prophetic sea-god), *Telluria* (Tellus, a Roman goddess personifying the Earth)
- **Morphological characteristics:** *Bacillus* (rod), *Streptococcus* (spheres with grape-like organization), *Helicobacter* (helical-shaped), *Campylobacter* (curved rod), *Clostridium* (greek word for *spindle*)
- **Biochemical characteristics:** *Achromobacter* (has no pigment), *Acinetobacter* (non-motile), *Chromobacterium* (produces pigment), *Anaerococcus* (strict anaerobe)
- **Geographical (e.g., site of first isolation):** *Budvicium* (Latin name of the city České Budějovice where the bacterium was first isolated), *Hafnia* (old name for Copenhagen), *Orientia* (the Orient, the area where the organisms are widely distributed), *Sinorhizobium* (which lives in a root in China)
- **Distribution:** *Aerococcus* (air), *Enterococcus* (gut), *Coprococcus* (feces), *Leptotrichia* (fine hair [of rabbits])
- **Disease-causing capability:** *S. pneumoniae* (pneumonia), *Vibrio cholerae* (cholera: watery diarrhoea), *N. meningitidis* (meningitis), *P. multocida* (lethal to many)
- **Institutions:** Centers for Disease Contol and Prevention (CDC): *Cedaceae*, Armed Forces Institute of Pathology (AFIP): *Apifia*

Changes at higher taxonomical levels (e.g., at orders and classes) are obviously much less likely to occur than in lower units, therefore one of the most common conventions on denominations is that the family name is based on the name of the most typical genus (i.e. a type species) in its domain [29-36]. A typical example is the *Legionellaceae* family, where the most characteristic genera is the one containing the *Legionella* species, namely *L. pneumophila*, the causative agent of Legionairre's disease. In contrast, for *Enterobacteriaceae*, *E. coli* is considered the type species, but the family

is not called *Escherichiaceae*; instead, due to the anatomical localization of most of these bacteria in the gut flora, they are classified in *Enterobacteriaceae*. Interestingly, the family *Enterobacteriaceae* contains a genus called *Enterobacter*, however, if one of the members of this genus would be the type species in the family, it would need to be called *Enterobacteraceae* [29-37]. The correct writing of bacterial taxonomical designations are also strictly defined by this convention, e.g. names of the species, genera and family are written in *italics*, however, at higher taxonomical designations, this is not done [29-36].

The use of abbreviations is also common in the literature and the routine clinical practice. Although there are official (defined in the Bacterial Code) three-letter abbreviations for a variety of bacterial genera (e.g., *Acp.* for *Acidophilum*, Rsc. for *Roseococcus*) to ease correspondences regarding anoxygenic phototrophic bacteria, other "real word" examples include mosaic terms derived from names of bacterial groups (e.g., GAS: Group A *Streptococcus*; ESKAPE: *Enterococcus faecium*, *S. aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *P. aeruginosa*, *Enterobacter* spp.), therapeutic recommendations (e.g., MRSA: methicillin-resistant *S. aureus*) or public health significance (e.g., MSTM: multidrug-resistant *Stenotrophomonas maltophilia*, MDRAB: multidrug-resistant *A. baumannii*) [27, 38-43]. It must be noted that in medicine (especially as far as the clinical microbiologist-physician relationship is concerned), the use of commonly known names is preferred, which are not subject to change (irrespective of taxonomic changes), so that the doctors reading the reports, e.g., of a susceptibility test can comprehend them [29-36].

3. Laboratory methods used in bacterial taxonomy and identification

The discipline of *classification* refers to the act of arranging bacteria into these group or taxa, based on their evolutionary relationships and similarity [44]. In the early days of bacterial taxonomy, the basis of classification was solely on the determination of microscopic morphology and phenotypic characteristics, which could be observed by a light microscope or by organoleptic analysis in liquid or solid media [11]. Later on, this was complemented by the detection of the presence or absence of various enzymes, such as coagulase (differentiates between coagulase positive *S. aureus* and coagulase-negative *Staphylococcus* species), catalase (among other things, it differentiates between *Staphylococcus* and *Streptococcus* species), oxidase (aids in the differentiation of non-fermenting Gram-negative bacteria, e.g., *Pseudomonas* and *Acinetobacter*), urease (among other things, it differentiates between *Ureaplasma* and *Mycoplasma* species) and the study of the use of different sugars (i.e. their oxidative or fermentative breakdown) [45]. For a very long period of time, these biochemical tests were the mainstay of *identification* in clinical bacteriology. Identification may be considered as *applied taxonomy*, during which microbiologists determine whether a particular isolate belongs to a recognized taxon (i.e., genus, species or subspecies) [1-3]. One of the main utilizers of bacterial identification in medicine is the field of clinical microbiology (where bacterial pathogens are identified from various clinical samples to establish the patient's illness and to guide targeted antimicrobial therapy) and public health (the follow-up of outbreaks caused by bacteria), however, companies involved in pharmaceutical research, biotechnology, forensics are all relevant stakeholders [21].

In the last several decades, pronounced changes were brought about in bacterial taxonomy, due to the introduction of nucleic acid-based and molecular techniques, thus making phenotypic methods less and less relevant [46]. These methods have demonstrated that genotypic/phylogenetic relatedness does not necessary correlate well with phenotypic attributes, such as a Gram-staining pattern, microscopic morphology, oxygen-tolerance or fastidious growth characteristics [47]. These molecular methods include comparison of DNA-denaturation or melting temperatures (T_m), characterization of GC (guanine and cytosine) ratios of bacterial DNA, DNA-DNA and DNA-RNA hybridization, pulse-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), average nucleotide identity (ANI), MALDI-TOF MS, 16S rRNA gene and whole genome sequencing (WGS) and next-generation sequencing (NGS) [48-50]. The use of these methods in increasingly prevalent not only in classification, but also in identification thus, revolutionizing the field of microbiology in the process [48-50]. The prevalence of their use is mainly determined by their accuracy, robustness and their price. Based on the abovementioned methods, two bacteria are considered to be the same species, if their nucleotide sequences are at least 70% identical, and the difference between their T_m values is less, than 5% [47]. The analysis

of the GC ratio (G+C content) in genomic DNA is also a suitable taxonomic method: the GC ratio is the most variable in prokaryotic genomes (20-80%); however, in strains of a specific species, the GC content is shown to be constant. It is basically defined as percentage of the G and C amino nucleotides in the bacterial genome, which was frequently used for the division of various bacterial genera (e.g., staphylococci and micrococci highly resemble each other, based on phenotypic and biochemical characteristics, however, the difference in their GC ratios has been shown to be pronounced [~30-40% vs. ~65-75%]) [51]. DNA–DNA hybridization was considered the gold standard for decades: genomic hybridization allows for the measurement of the degree of similarity between two genomes; this technique is very useful in the differentiation of closely-related bacterial species [52,53]. In contrast, DNA-RNA hybridization is useful in the genetic analysis of two phylogenetically distant bacteria: this is possible, because ribosomal RNA (rRNA) is transfer RNA (tRNA) only represent a minor portion of bacterial genes, which evolves in a slower pace (i.e. they are more conserved), compared to other genes coding for proteins [52,53]. Currently, various nucleic acid sequencing methods (of which, WGS and NGS are one of the most modern) represent the top-tier methods for bacterial classification and the comparison of genomic structures [48-50]. Nucleic acid (DNA and RNA) sequencing is another molecular characteristic that helps directly compare the genomic structures. The sequencing of 5S rRNA (from the 50S prokaryotic ribosomal subunit), 16S rRNA and 16S rDNA (from the 30S prokaryotic ribosomal subunit) has received the most substantial attention [47-50]. In fact, current recommendations state that for the submission of a novel species, the performance of MLST or sequencing (to characterize genomic relatedness) and the submission of a preferably full-length 16S rRNA gene sequence are recommended [47].

Nevertheless, it is now well-known that the phenotypic as well as the genotypic characteristics of bacteria may be subject to change due to exogenous genetic material (i.e. conjugation, transformation and transduction), which entails the transfer of plasmid DNA from one species/genus/family of bacteria to another. In reality, these properties may also be useful to characterize relations between different bacterial taxa [47-50]. *E. coli* species conjugate well with *Salmonella* and *Shigella* species (which are more closely related taxonomically), but not with members of the genera *Proteus, Providencia* or *Enterobacter*. Similar results were found in transformation studies on *Rhizobium*, *Micrococcus*, *Bacillus* and *Haemophilus* species, showing that transformation events more frequently occur with different species of the same genera (smaller genomic variation), compared to species of different genera (larger genomic variation) [47-50].

Table III *Examples of bacterial species undergone taxonomic revisions in the last 20-year period*

Previous taxonomic designation	Current taxonomic designation
Actinobaculum schaali	*Actinotignum schaali*
Actinobacillus actinomycetemcomitans	*Aggregatibacter actinomycetemcomitans*
Bacteroides forsythus	*Tannerella forsythia*
Bacteroides gracilis	*Campylobacter gracilis*
Bacteroides melaninogenicus	*Prevotella melaninogenica*
Bacteroides pneumosintes	*Dialister pneumosintes*
Borellia burgdorferi	*Borelliella burgdorferi*
Clostridium difficile	*Clostridioides difficile*
Enterobacter shakazakii	*Cronobacter shakazakii*
Enterobacter aerogenes	*Klebsiella aerogenes*
Enterobacter gergoviae	*Pluralibacter gergoviae*
Enterobacter amnigenus	*Lelliottia amnigena*
Eubacterium lentum	*Eggerthella lenta*
Klebsiella pnuemonaie ATCC 700603	*K. quasipneumoniae subsp. similipneumoniae*
Peptostreptococcus micros	*Parvimonas micra*
Propionibacterium acnes	*Cutibacterium acnes*
Streptococcus tigurinus	*S. oralis subsp. tigurinus*
Wolinella rectus	*Camplyobacter rectus*

4. Practical relevance of taxonomical changes

After the official recognition and acknowledgement of taxonomic alterations or a revised nomenclature, significant changes may occur in the everyday practice of physicians and microbiologists dealing with infectious diseases, epidemiologists, university educations and other relevant stakeholders [47]. Changes in bacterial taxonomy, and nomenclature is usually greeted with conservatism and resistance among taxonomists, microbiologists, healthcare-professionals and scientist alike, for the simple reason that nobody likes change [1-3,21,47]. The applications of taxonomic changes can be broad ranging: they may impact the clinical care of patients, through variations in choosing the appropriate antimicrobial susceptibility testing standards or data interpretation, or even their clinical relevance and epidemiology (commensal/colonizer/pathogen). These changes also affect companies supplying laboratories with testing equipment and software (i.e. the laboratory information system or LIS) and even administrative stakeholders (e.g., accreditation services, conformity with legal documentation); of course, the clinical relevance of these changes is also relative to the isolation frequency and invasiveness of the abovementioned bacteria [33,36,37,43,44,47]. Some recent changes of interest in bacterial taxonomy are discussed below and presented in *Table III*.

Gram-negative bacteria (especially ones representing gut bacteria) have seen a plethora of taxonomic revisions since the beginning of the 21st century. Among other things, some *Vibrio* species have been reclassified into the genera *Photobacterium* (e.g., *P. damselae*) and *Grimontia* (*G. hollisae*), and the phylogenetically heterogenous members of the *E. cloacae* complex has been reassigned to the genera *Kosakonia*, *Lelliottia*, and *Pluralibacter* [54-56]. Another relevant change was the one affecting the genus *Salmonella*, where only *Salmonella enterica* strains remained in the species status, while other serovariants (e.g., Enteritidis, Typhimurium, Typhi) are no longer recognized on the species level, therefore their names should no longer be italicized [6,57]. However, one of the major taxonomical changes affecting Gram-negative bacteria (and subsequently, the medical community) is the recent reclassification of the family *Enterobacteriaceae* into the order *Enterobacterales*, containing seven distinct families (namely *Enterobacteriaceae*, *Erwiniaceae*, *Pectobacteriaceae*, *Yersiniaceae*, *Hafniaceae*, *Morganellaceae* and *Budviciaceae*) based on recent phylogenetic analyses [58]. Other suggestions include the differentiation of all *Burkholderia* species into two distinct groups: the genus *Burkolderia* would contain the human pathogenic species, while a newly designated genus *Paraburkholderia* would hold the non-pathogenic species to humans [59]. In contrast, it was proposed that the genera *Chlamydia* and *Chlamydophila* (containing *C. pneumoniae* and *C. psittaci*) should be fused together, eliminating the latter genus in the process [60].

Pronounced taxonomic changes have also occured regarding anaerobic bacteria in the last 30-40 years [16]. The restriction of the genus *Bacteroides* to *B. fragilis* and related species has led to the relclassification and transfer of numerous species to the genera *Prevotella* and *Porphyromonas* (based on pigmentation, bile-sensitivity and saccharolytic properties) and the introduction of novel genera [61-64]. Marked changes have also occured in the field of Gram-positive anaerobic cocci with the introduction of novel species, such as *Finegoldia*, *Parvimonas* and *Peptinophilus*, based on phylogenetic analysis [16,65-66]. *Eubacterium* species were also subject to taxonomic revisions, leading to the introduction of novel genera, such as *Slackia*, *Pseudoramibacter*, *Mogibacterium*, *Eggerthella* and *Cryptobacterium* [16,65]. Perhaps the most controversial taxonomic revision occured regarding the causative agent of antibiotic-associated diarrhoea and pseudomembranous enterocolitis, namely *Clostridium* (*Clostridioides*) *difficile*, which may be considered as the prime example why taxonomic changes have to be carefully considered [67-68]. After the proposal to restric the genus *Clostridium* to *C. butyricum* and other related species, it was found that *C. difficile* was phylogenetically closest to *C. mangenotii* with a 94.7% similarity, however, this species was located in the family *Peptostreptococcaceae* [69]. This would have lead to a nomenclature revision of *C. difficile* as *Peptoclostridium difficile*; however, due to the significance of this pathogen in nosocomial infection and as a public health threat, a lot of energy, time and money was put into the education of the public and healthcare professionals around the globe, regarding the dangers of "C. diff" (as it is colloquially known) and CDD/CDAD (*C. difficile*-associated diarrhea), with educational campaigns, fliers, books and so on [16, 67-69]. The proposed taxonomic change would have put forth issues in this educational

campaign (a sudden change of "*C. diff*" to "*P. diff*" and CDAD to PDAD and so on); for this reason, the reclassification as *P. difficile* was rejected, instead, a novel genus *Clostridioides* gen. nov. was proposed for *C. difficile* (now *Clostridioides difficile*) and *C. magnerotii* was also reclassified to this new genus; therefore previously used, colloquial designations for this pathogen (*C. diff*, CDD/CDAD) also remained valid [70,71].

5. Conclusions

Taxonomy is concerned with the classification of living organisms, which operates in three distinct domains, namely classification, nomenclature and identification. Compared to the taxonomic trends in the 19th century, current methods and technologies allow for more detailed phylogenetic analyses, leading to the description of a tremendous amount of novel bacterial species and the re-classification of several already described bacteria. This 'explosion' in microbial taxonomy (further aided by the developments in bacterial systematics) presents an everyday challenge to medical professionals (e.g., clinical pharmacists, physicians and nurses), pharmaceutical scientists and stakeholders in healthcare. However, the up-to-date knowledge on bacterial taxonomy is important as it may significantly impact the everyday practice of these healthcare professionals. This is especially true for scientists who use various bacterial strains for screening of antimicrobial activity of various compounds or utilizing any kind of bacterial model system during laboratory assays. The aim of this paper was to aid the abovementioned healthcare professionals to navigate through the 'maze' of bacterial taxonomy, to aid in finding authentic information regarding the description of taxonomic changes and to present some examples of changes in bacterial taxonomy which proven to be clinically significant.

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7. Competing interests

The author declares no conflict of interest, monetary or otherwise.

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How to design an education programme for patients with chronic obstructive disease?

Learnings from a pilot community pharmacy based project to evaluate patient attitudes

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Abstract
Introduction: *COPD is a debilitating disease and a major death cause by 2020. Our current knowledge of the opportunities of patient education in the community pharmacy is growing, though yet limited.*
Objectives: *(1) To assess the potential of a patient education programme in a Hungarian pharmacy in a pilot setting, (2) to understand patient attitudes and gather insight for the development of an education project, (3) to create a sustainable local good practice.*
Methods: *We invited patients with a confirmed COPD diagnosis to take part in an in-depth interview, and to assess their symptoms. Later on, we offered them tailor-made education to learn about their attitudes to create the guidelines for optimal content.*
Results: *Key elements of the education content should focus on the desire of active life, improve poor adherence, teach about reliever and maintenance therapy and emphasise the chronic component of the disease. Smoking cessation should be fostered, whilst patients would benefit from proper breathing techniques and posture.*
Conclusion: *Hungarian patients may prefer to get fast and basic education in the community pharmacies. The ideal education content should include pathophysiology, signs and symptoms, treatment options, explanation of medication, inhaler use, smoking cessation and breathing techniques.*

Keywords: *COPD, patient education, ideal content, patient attitudes and perceptions*

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a respiratory disorder that negatively affects lung function [1], deteriorates quality of life and disables patients to perform their daily duties [2]. It is intercalated by exacerbations, acutely worsening periods, which may require hospital care [1]. The more exacerbations a patient has, the faster their status drops [3].

By 2020, COPD will be the third most common death cause all over the world [4], which poses a severe burden on societies [1]. At the community level, it is our common interest to help patients with this condition, to foster smoking cessation and support their disease management [5]. On top of the effects on the quality of life of the patient, COPD results in a higher rate of utilisation of health services, such as medication, prolonged hospital stay due to exacerbations, as well as the subsequent need of rehabilitation.

Current research trends in COPD disease management indicate that our knowledge of the community pharmacy setting is growing [6, 7], though still not abundant [2, 8]. As of Hungary, we have been unable to identify significant body of research related to COPD patient education in the community pharmacy setting.[1] Some examples of education in pulmonology centres have been found. These circumstances drive to our attention to an interesting, yet less studied area, for which we have implemented a pilot project in collaboration with the Inczeffy Pharmacy and Medical Centre in Göd.

2. Objectives

1. The primary objective was to assess the opportunities of COPD disease management in the community pharmacy in a *pilot* setting by in-depth interviews with current COPD patients who are also future subjects of the education programme.

[1] Our Pubmed based advanced search (COPD AND Hungary AND patient education), also checking all relevant articles about (COPD AND Hungary), has not revealed any publication on community pharmacy based education of Hungarian COPD patients (performed by one reviewer, 31 Aug 2019).

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Table I *Interview methodology guide corresponding to the objectives of the study*

Objective	Topic	Sample questions
1	In-depth interviews to understand patient attitudes, perceptions	How do you feel about your disease?
		What disturbs you most in COPD?
		What would you like to do if you could breathe freely?
		Do you think your condition will get any better?
		What does a regular day of yours look like?
2	Explorative interviews for educational content & insights to adherence	Do you have any questions related to your condition? / What would you like to know about it?
		What have you heard about it?
		Can you tell us how to use these inhalers?
		What would you like to change about the condition?
		Do you believe that you / the doctor / your educator can make a change? How?
3	Creating local good practice in Göd	How can the pharmacy staff be engaged?
		How can we engage local stakeholders to keep up with the project?

2. The secondary objective was to understand patient attitudes, perceptions, fears, beliefs that affect the everyday life of patients. This is highly valuable input to the education programme, because it sets the guidelines for the educational content, whilst providing major insights into how patients relate to their disease.
3. The tertiary objective was to create a local good practice and to investigate how the community pharmacy pillar of integrated care can work in the context of a small town in Hungary.

3. Methods

3.1. Study context

Göd is a medium-size city with around 19,800 inhabitants [9]. According to the Rotterdam Study [5], COPD prevalence on an overall basis is 4.6%, so 5% seems to be a rationale approximation in a European setting [4]. This means that the overall COPD population of Göd should be around 95 people, out of which, N=6 were included in our study (7%). Inczeffy Pharmacy is a central and well-known community pharmacy in the city, and it has gained an innovative reputation due to their previous commitment in previous patient education programmes.

This explorative study joins methodologically a series of studies. Besides patient interviews in the pharmacy and the pulmonology outpatient centres, pulmonologist expert opinion was assessed to develop an education programme for the COPD patients. Later on n=118 patients were provided education, and their quality of life and adherence changes were monitored in a longitudinal study with follow-up. The in-depth interview methodology, applied in this context, helped us explore the opportunities of patient education in the pharmacy, and also to lay more stress on the pulmonology centre pillar.

3.2. Inclusion of study participants

During dispensing medication, patients with the J44 ICD classification code were offered to participate in the pilot project. Altogether, patients were screened for 5 working days, 25 showed their interest, and 8 were willing to participate in the education session, and finally 6 patients showed up.

3.3. Interview methodology

We have performed exploratory in-depth interviews with the patients [10]. The structure of the interview has been designed to determine the key elements of a patient education programme; and to let them discover perception and therapeutic attitudes [11]. *Table I* provides an overview of this structure and an insight into the explorative questions. One interview lasted ca. 30 minutes. The methodology showed some mixed features with a loose semi-structured interview, since we primarily set the major topics we wanted to direct pa-

tients to. Altogether, interviewed them by using the active listening technique, which means that we concentrated on their thoughts and let them relatively freely talk about their condition. At the end of the interviews, patients participated in an educational session (prototype education) where they could ask freely about their doubts related to their disease. Their reactions were assessed before and after the prototype session.

3.4. Symptom assessment

We invited the patients to self-administer the COPD Assessment Tool (CAT),[2] which is a standard method to assess symptomatology in pulmonology practice [12]. CAT is an 8-item questionnaire to assess their symptoms on an ascending scale until 5, and scores range 0-40. Patients are considered symptomatic above 10 scores.

4. Results

The community pharmacy setting is a challenging venue for patient education. Originally, we intended to incorporate a comparative aspect to a longitudinal study which assesses the impact of education on quality of life and adherence; however, pharmacy patients turned out to be more willing to be educated in a one-time occasion than in a follow-up intervention. Consequently, the community pharmacy pillar to this study was halted, whilst the interviews provided patient insight to the education content we developed for the further course of the aforementioned study. The patients' desire to get pharmacist advice fast [2], seems to also appear in a German setting, which verifies the need for pharmacy disease management projects (patients who attended such sessions were enabled to use their inhaler significantly better, although this means 29% overall) [13]. This idea has been further developed in Japan, where pharmacist-led clinics were proven to improve quality of life [14].

4.1. In-depth interviews to understand patient attitudes, perceptions

Patients did not mention the textbook like symptoms (sputum secretion, dyspnoea and coughing) on the first instance; however, they always relate to real-life actions they would like to perform: to play tennis, to play with the grandchildren and to paint. Falsely, they identify their illness as asthma, and they see no relation to smoking (but one patient). They get used to the symptoms, and they mostly perceive them as "they come by age", "they are natural"; thus, they accept their conditions. These activities should be used when developing the patient profile for the education content; additionally, we should use the patients' own words to help them memorise its content. *Figure 1* summarises the results for the education content.

4.2. Educational content and insight to adherence

None of the patients was able to identify the reliever drug; even the most asymptomatic patient used it every morning, in an inadequate dose. Once-daily inhalers were preferred, since one occasion is the frequency everyone declared as adequate for the treatment of the disease. One patient reported a willingness to apply alternative methods, namely she was interested in herbal drinks as a possible treatment option. The patients were unaware of the inhalation therapy they used; hence, there was no apparent fear of steroids. Based on their CAT scores, all patients were symptomatic, which implies that they are either not compliant, or do not have the right medication.

The interviews reveal a truly diverse image of COPD attitudes. The main symptoms are feeling powerless and the lack of physical performance. Two patients mentioned repeatedly their disability in moving and taking the stairs, and one patient was completely confined to oxygen therapy.

The lack of adherence is a variant which was challenging to get the patient talk about. Finally, they all confessed that they either do not take their medication in the prescribed manner, or make changes to treatment regimens upon self-judgement. The better they feel, the less they take the medication.

4.3. A local good practice

Since the project was well-accepted, a local epitome has been created in the Inczeffy Pharmacy. It is our ambition to keep this project going, and in order to maximize the impact our our research, interested pharmacies are invited to collaborate throughout Hungary.

Our results are in line with review articles in COPD patient education in the community pharmacy setting [15, 16]. The primary roles of the pharmacist should be to assess the current symp-

[2] We fully complied with the guidelines at https://www.catestonline.org, and we solely used the licence for academic research purposes.

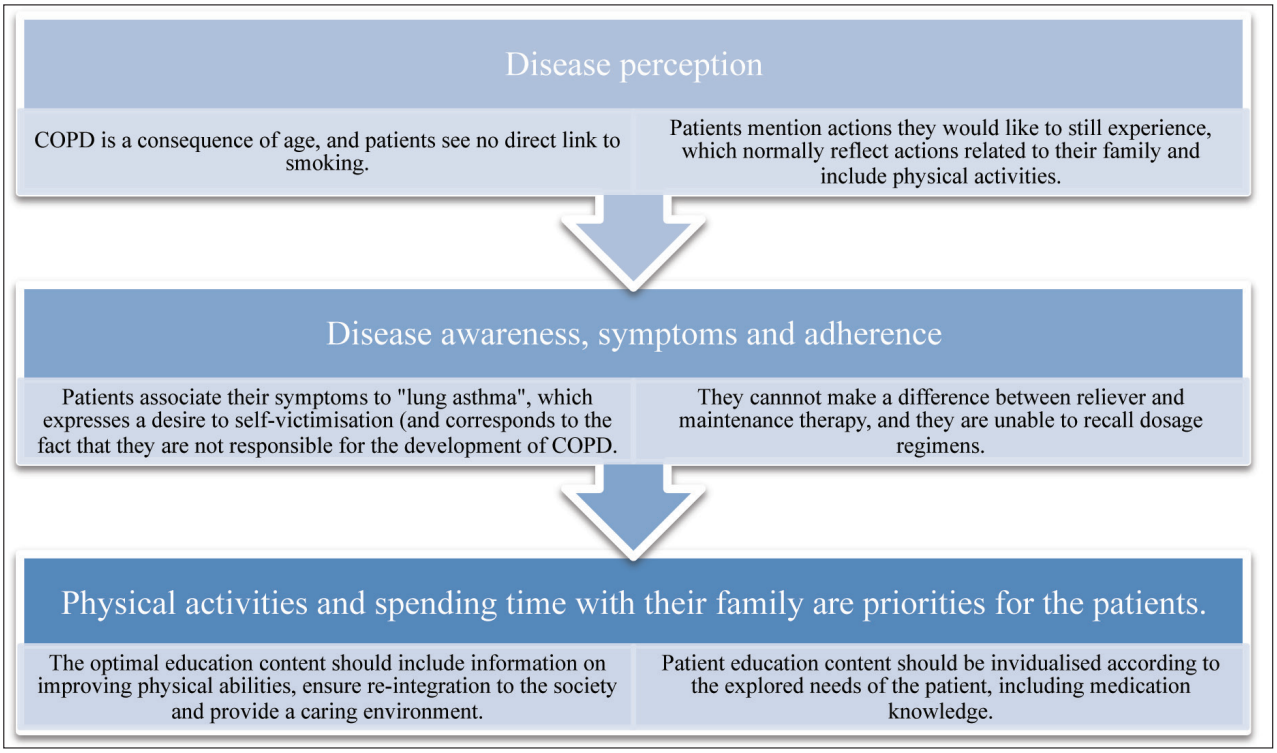

Figure 1 *Major patient perceptions & attitudes and their implications for the development of education content*

toms and improving smoking habits, inhaler technique, dosage and medication use, and the provision of materials to support these activities. Based on the input provided by this study, we can state that the education materials created reflect international good practice.

5. Discussion

While designing the education content, I identified similar body of research which describes an educational project with n = 62 patients with moderate to mild COPD (<70 years of age), who participated 2x2 hour weekly session, with 1 week gap fashion [17]. Education was performed through a 19-page booklet with information on self-assessment and disease management. Oral sessions included education on the respiratory obstruction, anti-obstructive medication, exacerbation prevention, self-assessment and self-management, and physiotherapy. This is a definite similarity with our research, since the major elements of the education are overlapping. In line with our results, the implication of this is that we should make sure that patients understand that COPD is a chronic condition, and medications should be taken; also in case they feel right. Interestingly, SMART dosage is available in the case of certain drugs, so this is another aspect that should be included in the education content.

Asking the patient and the caregiver at the same time can draw our attention to new discoveries [18]. For the conceptual perception assessment, it is worth including patient and caregiver interviews to map the needs of the most important stakeholders of COPD care in Hungary. This current study evaluated the importance of pharmacist care and the educational opportunities in the community pharmacy setting. It is important to note that patients originally did not associate lung problems with the pharmacists' relevant knowledge, though confidence was built up once they realized that their doubts were correctly addressed.

In order to keep up with this spirit and to build the educators' reputation in the project, we identified the following key benefits of employing a pharmacist in the project, along with [19]:

1. primary prevention: campaigns, lifestyle counseling, awareness raising;
2. early diagnosis;
3. management and ongoing support: pharmacist care, information on inhalation device use, disease outlook, dosage, self-management of the disease;
4. overview and follow-up: monitoring adherence

and device use. This connects to the content of the community pharmacy pillar of our education, and we considered these points to define the potential role of the pharmacist in our education project.

An important methodological point is highlighted: due to the heterogeneity of the studies, it is very hard to set up the optimal education, based on the publications reviewed, but it may be tailormade [20]. Finding the right balance between fixed content to keep measurements intercomparable is a methodological prerequisite, whilst personalization seems to bring the most benefits to the patient.

Taking the matter of standardization of education content to a national level, data providers in Germany present such diversity that prefer not to compare [21]. The study confirms that these education projects should be either aligned individually or require a higher level of coordination for initiation. The most common errors that were found in 46 of the 95 programs are as follows: evaluation of program success, inadequate transparency of cost data and the lack of the same in quality of life interventions. It seems clear that success rates should be defined, although there are no consensus or an established method [22]. A prerequisite for the achievement of success indicators is that the patient is actively involved in the therapeutic process and has an individual action plan for the self-management of the disease [23]. Although very softly, [24, 25] also affirm that a caring environment, nice and competent words initiate the self-management process in the COPD patient. That is why we considered it supremely important along this study to keep patients motivated, especially by providing answers to all additional questions, once the sessions were over, and the education with fixed content was delivered.

A holistic summary of patient education opportunities [26] include printed *brochures*, recorded videos and audio-visual materials, self-education, self-monitoring, *self-directed therapy, patient involvement in therapy*, patient interviews on side effects, *organization of self-help and therapy groups*, telemedicine, computer and internet patient information, targeted interventions to improve health literacy in disadvantaged groups, and targeted media campaigns. The methods mentioned here depict another process: media and telemedicine should be the future direction, though currently we have not identified this need from our patients, apparently it would have been a disadvantage at this phase. Currently, we applied the methods in italics, and focused on the major benefit of the education sessions highlighted by the interview subjects, which is, personal care and assistance throughout the project.

A Canadian patient education project [27] covered adherence, inhalation techniques, health-related quality of life, and the use of health resources such as drug therapy and COPD exacerbations. Content included explanation of the current therapy, dosage, administration, patient expectations, duration of therapy, and potential outcomes, and follow-ups and improved inhaler use. By the "teach it back" strategy, understanding the components of adherence caused by a lack of knowledge and the patient's perceptions of the disease have helped to enhance adherence. The "teach it back" strategy that is also vastly recommended by our interviews, patients appreciated when their opinion was considered and they were included in the sessions by embracing their true self and attitudes.

An analog of this study [28] examined COPD self-management on n=176 patients, with the following education content: COPD status, medication, and respiratory training. Education should be structured to ensure that the measurements are inter-comparable. The education should consider the patients' capabilities, so that the content can be acquired, and it has cost-effective long-term effects: less frequent exacerbations result in decreased use of healthcare resources [29].

The Belgian PHARMACOP study [30] shares the methodology of [31] and they are very similar to the final study design of our investigations. Altogether, n = 734 patients were enrolled and followed for 3 months. Adherence to maintenance therapy and the use of inhalation devices were the focus of the study, and education was provided to patients at baseline and after 1 month. Both variables were significantly better in the intervention group, and a significantly lower number of hospitalizations were reported. Using the in-depth interview method, n=173 patients were reported that the absence of depression, comorbidities, and patient perception of the disease have a much greater impact on adherence than demographics or disease severity [28].

A major limitation for the first sight is the numbers of patients included in the study. In the setting of Göd, this means 7% inclusion of the whole population. In order to achieve the same power of significance, we would need to include 35,000 pa-

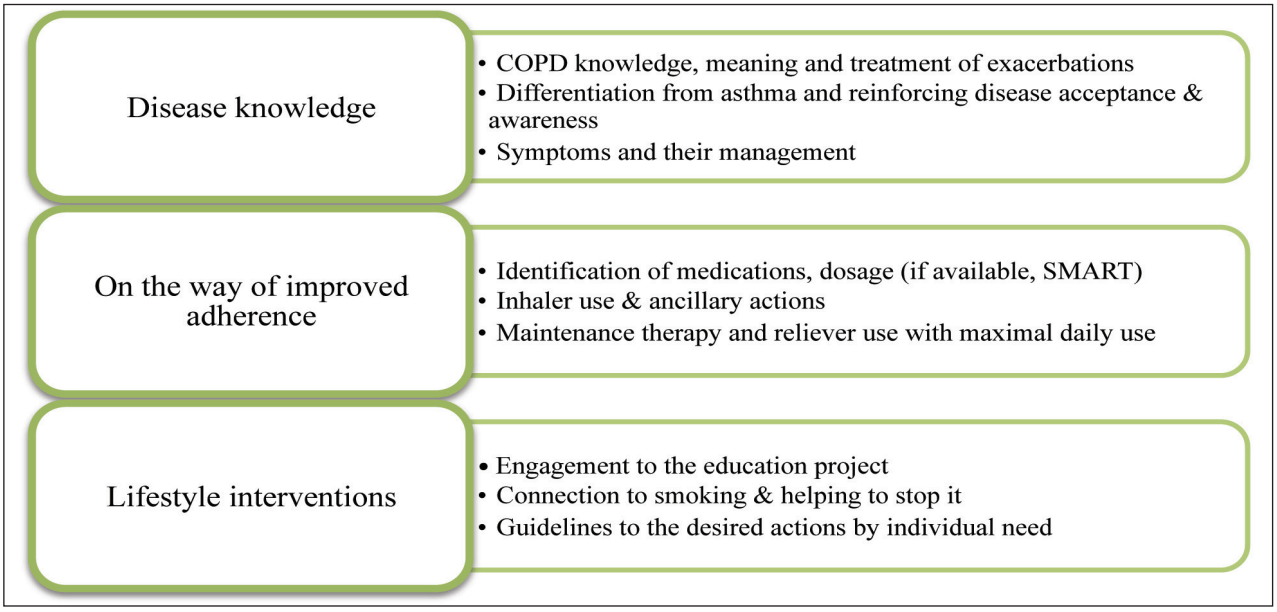

Figure 2 *Elements of the suggested education content by COPD patients*

tients for Hungary, considering 500,000 COPD patients in the country [5]. Consequently, this aspect is considered as a relative limitation; however, it is an obstacle of the general applicability of our conclusions.

6. Conclusions and caveats

We have developed an education content for COPD patients based on the input from patients from the community pharmacy. The education programme should reinforce physical activities, tailor-made to the current condition of the patient; furthermore, breathing techniques should be incorporated to avoid the abrupt appearance of breathlessness. Our relatively small sample demonstrated such a diversity (like the ladies' interest in alternative methods) that corresponds with patient beliefs described in the literature.

Patient interviews vastly reveal the missing concepts, lack of pragmatic knowledge and (non)-adherence data that should be tackled by in our education programme. A patient education project was piloted in the community setting, and we will do our best to ensure the scientific support to its continuation (hopefully this means that research objective III will continue on the long-term in Göd). Furthermore, this study has the benefit to envision the three key intervention areas that should be targeted by an education programme.

1. A relatively small number of COPD patients prefers the pharmacy to get education; however, those who come indeed, are motivated. Patients get used to COPD, and they accept "that this comes by age", "I cannot do this", though all of them mentioned an activity they still wish to pursue.
2. Active listening to patient needs is an effective way to reveal important insight [11]. Namely, asking semi-structured questions; and permitting them to talk about their issues helped us create the guidelines for such content they can benefit. Recording patients' words and phrases also helps us to put together an easy-to-understand material with catchy content. There is a need to expand the project, since once the patients decided to open up, they share all their experience; furthermore, these patient clubs are also in favour of their social inclusion and provide an opportunity to meet their peers.
3. Research objectives I and II have been also met, and our findings are summarised in *Figure 2*. This table also seeks to incorporate the patient insight to an ideal education content. Since the project was well accepted, a local epitome has been created in the Inczeffy Pharmacy.
4. It should be noted that the generalizability of our results is limited due to the small amount of patients included in the study, though this number represents 7% of the total COPD population of Göd. Consequently, our results can be primarily applied in similar setting of towns or smaller cities.
5. These results imply for the further development of research, that COPD patients prefer to take part in education more in the pulmonology out-

patient centre setting than the pharmacy. In order to meet this need, a larger number of patients were recruited from the latter setting.

7. Acknowledgement

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8. Competing interests

The authors declare no conflict of interest, monetary or otherwise.

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Standard operating procedure (SOP) for disk diffusion-based quorum sensing inhibition assays

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Abstract
Introduction: *The emergence of multidrug-resistant bacterial strains is a severe global health issue, which is worsened by the inability of new antibiotics. Virulence inhibition is one of the novel strategies that have been proposed to combat bacterial pathogens more effectively, without the risk of exerting selection pressure on these microorganisms. Inhibition of bacterial cell-cell communication (quorum sensing; QS) is a promising approach however, rapid and cost-effective screening for compounds with QS-inhibitory activity is not yet well-established.* ***Aims:*** *The aim of the present study is to determine the ideal experimental conditions for the disk-diffusion based QS-inhibitory assay with the most frequently used QS-signal molecule-producing and reporter strains.*
Methods: *In our study, the effects of growth characteristics, incubation time, temperature and the used culture media were studied on the used bacterial strains and results of the disk-diffusion based QS-inhibitory assay.*
Results: *Based on our results, the ideal experimental setting includes a modified Luria-Bertani medium (LB*; complemented with nutrients and microelements), incubation at room temperature (25 °C) for 48 hours before the reading of results, where the density of the starting inocula has less influence of the results of the assay.*
Conclusion: *Establishing standard operating procedures (SOPs) is a way to help carry out various operations, aiming to increase precision and efficiency. Adherence to the experimental settings defined based on our results may aid in improving the reproducibility, comparability and reliability of results obtained by this method.*

Keywords: *standard operating procedures, quorum sensing, QS, disk diffusion, Chromobacterium, violacein, Serratia, prodigiosin, Agrobacterium, pigment*

1. Introduction

Bacterial infections are still major factors of morbidity and mortality in both developing and developed countries worldwide therefore, antibiotics should be considered medicines of special importance [1]. In addition to being the causal therapy of often life-threatening infections (e.g., sepsis), antibiotics have paved the way for the development of many medical specialities (e.g., complex surgical procedures, organ transplantation, cancer chemotherapy, neonatology) [2, 3]. The continuous emergence of resistance bacterial strains (especially multidrug-resistant [MDR] pathogens) is becoming a severe global health issue [4,5]. One of the best ways to combat antimicrobial drug resistance is with the development of novel antibiotic drugs (which was the standard course of conduct during the 1960-1980's), nowadays however, the pharmaceutical companies are struggling to keep up with the continuous and detrimental developments in resistance trends [6,7]. The scarcity of new agents in the 'antibiotic pipeline' could be attributed to economical (antibiotics have high developmental costs and modest returns of investment, development of drugs for chronic illnesses and cancer is much more lucrative), clinical (the difficulties of arranging and tracking clinical trials) and microbiological (the emergence of resistant strains is inevitable) characteristics [8,9]. For this reason, no novel broad-spectrum agent has been developed since the discovery of the fluoroquinolones in the 1980's, while the dynamic increase in the prevalence of resistant isolates has been reported worldwide [10].

Due to the scarcity of available therapeutic options, novel strategies have been proposed to combat bacterial pathogens more effectively [11,12]. One of these strategies is combination therapy with the use of existing antibiotics, however, except for some well-defined clinical situations, the clinical utility of antibiotic combinations has been controversial, in addition to their costs for the healthcare infrastructure [13]. Another possible therapeutic alternative is to utilize adjuvant compounds (together with antibiotics) during therapy [12,14]. These antimicrobial adjuvants are classified to two distinct categories: Class I adjuvants affect the microorganism, while Class II adjuvants

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affect the cells of the host. Class I adjuvants include examples, such as β-lactamase inhibitors (which have been successfully used in therapy for many decades against various β-lactamase-producing pathogens), bacterial efflux pump inhibitors (e.g., phenylalanine-arginine β-naphthylamide (PAβN, although these compounds only have relevance in theoretical models and experimental settings for now, because most of them are toxic in the efflux pump inhibitory concentrations)) and modulators of bacterial membrane potential (e.g., loperamide) and compounds inhibiting bacterial toxin synthesis or neutralizing antibodies (e.g., bezlotoxumab against the toxins of *Clostridioides difficile*) [14,15]. Class I adjuvants may be useful, as they may in theory, make old antibiotics useful again, that have already been eliminated from clinical practice due to their widespread resistance. Class II adjuvants are usually compounds enhancing the immune response of the host organism against the foreign invaders (e.g., streptazolin, as a stimulant of macrophages and natural killer-cells) [14]. Another promising approach to fight bacterial infections is the use of virulence inhibitors: these compounds do not affect the viability of these cells, instead, they inhibit the synthesis or expression of bacterial virulence factors, which are key in their pathogenesis [16]. The potential advantage of these agents is that the selection pressure (and consequently, the chance of resistance development) is expected to be much lower [17].

Quorum sensing (QS; also called autoinduction) is a chemical-sociobiological mechanism of communication, during which bacteria can regulate the expression of specific genes (which are important for benefits in fitness and reproductive success in their niche), in response to the density of cells in the surrounding environment [18,19]. This includes the detection of signal molecules produced by surrounding cells and also self-produced signals (leading to positive feed-back; these autoinducers (or bacterial 'pheromones') diffuse into the specific niche, where their concentrations grow proportionally with the number of bacterial cells [20]. If the concentration of these signal molecules reaches a critical concentration (corresponding to a critical population density), these signal molecules initiate the transcription of various target genes [18-20]. Quorum sensing was first described in the marine bacterium *Vibrio fischeri*, a symbiont in the light organ of some marine animals: if bacteria reach a threshold population density, genes encoding bioluminescence are expressed [21]. QS mediates the expression of various features important in bacterial physiology and virulence, leading to phenotypic changes: expression of toxin genes (e.g., toxic shock syndrome toxin in *Staphylococcus aureus*, elastase in *Pseudomonas aeruginosa,* protease in *V. cholerae*), bacterial secretion systems (e.g., *Salmonella species*), efflux pumps (e.g., *P. aeruginosa*), biofilm-production (e.g., *P. aeruginosa*, *Acinetobacter baumannii*), induction of bacterial competence (*Streptococcus pneumoniae*), motility (e.g., *P. aeruginosa*) and production of pigments (e.g., *Chromobacterium violaceum, Serratia marcescens*) [22-27]. Quorum sensing has also been implicated in facilitating the spread of antibiotic-resistance genes [18,28-30].

QS signal molecules include a wide range of compounds with distinct structural characteristics [18-20]. In Gram-negative bacteria, derivatives of L-homoserine lactone (acyl-HSLs or AHLs) are the most prevalent, while in Gram-positive bacteria, peptide-based signal molecules (autoinducing peptides, AIPs, which are post-transcriptionally

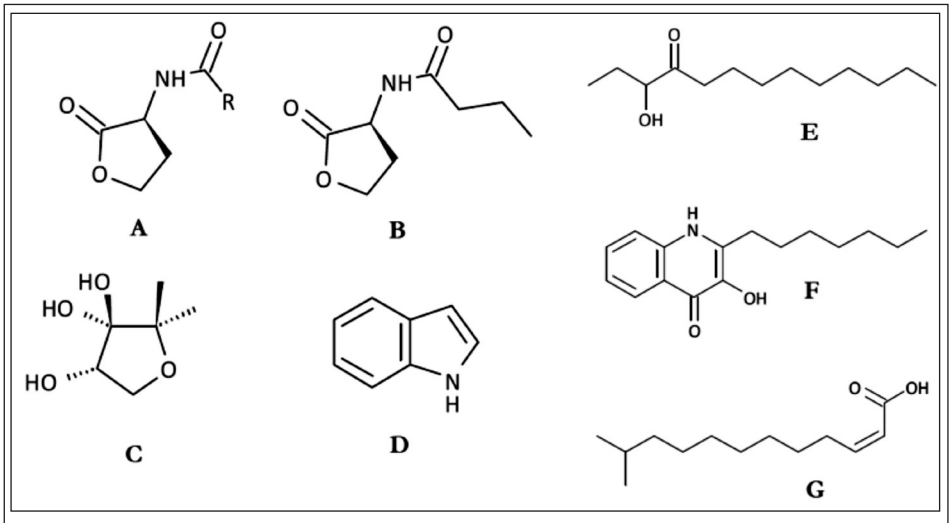

Figure 1 Examples of quorum sensing signal molecules (autoinducers) *[18-27, 31]*
A: *Acyl-homoserine-lactones (AHL);* **B:** *Butanoyl-homoserine-lactones (BHL);* **C:** *Autoinducer-2 (AI);* **D:** *Indole;* **E:** *Cholera autoinducer (CAI-1/V. cholerae);* **F:** *Pseudomonas quinolone signal (PQS);* **G:** *Diffusible signal factor (DSF)*

modified small peptides) are most frequently detected. Some signaling molecules are detected by both groups (e.g., AI-2, a derivative of dihydroxy-2,3-pentanedione) (for examples, see *Figure 1*) [18-23]. Although the signal molecules may differ, the consequent mechanism of activation caused by these molecules is very similar in all bacteria [18-23]. In Gram-negative bacteria AHLs may be characterized by the nature and length of the substitution at the 3-carbon position, and the presence of unsaturated chains within the acyl chain [18,19,31].

The elimination or inhibition of QS-signal transmission is termed quorum quenching (QQ) [32]. This may be a consequence of inhibition of autoinducer-synthesis, degradation of signal molecule or through the use of signal-antagonists, inhibiting the sensing of these signal molecules by the relevant bacteria [19,31,32]. It is no surprise that many organisms possess enzymes with activity against such signal molecules (e.g., the human paraoxonase (PON, a lactonase) can also degrade AHLs). Synthetic compounds (inhibition-based QQ) or enzymes (degradation-based QQ) do not kill pathogenic bacteria, instead they inhibit the signal transduction mechanisms important in the expression of their virulence determinants (thus, disarming them) [14,16,19,31,32]. QQ-compounds may be considered as potential therapeutic alternatives in the treatment of bacterial infections, as they are capable of eliminating the disease-causing capacity of bacteria, without the risk of rapid resistance development [18-20, 31-33]. Several *in vitro* and *in vivo* model systems have been developed for the qualitative and quantitative evaluation of a compounds QS-inhibitory activity: these methods may include the use of Petri-dish or microplate-based colorimetric methods, molecular biological techniques (e.g., polymerase chain reaction), animal models and transgenic constructs [34-36]. Disk diffusion is a simple method for screening the susceptibility of various microorganisms against drugs/candidate molecules: it is user-friendly, and there is a lot of experience accumulated due to its use in routine clinical microbiology. Disk-diffusion based QS-inhibitory (DDBQSI) assay utilizing QS-signal molecule producing strains and signal molecule-reporter strains (e.g., *Agrobacterium*, *Chromobacterium*, *Pseudomonas*, *Serratia* and *Vibrio* species) is the most frequently used method [36-40]. The advantage of this method is its simple execution, the high-throughput nature and its usability in resource-scarce settings [41]. Nevertheless, there are many different and conflicting experimental protocols described for DDBQSI-assays in the literature, which makes it difficult to evaluate and compare published results. Additionally, the reproducibility of positive results still represents an important challenge for laboratories, because growth characteristics and pigment production by these bacteria is also subject to some additional factors [37]. The aim of the present study is to determine the ideal experimental conditions (i.e., incubation time, temperature, culture media) for disk-diffusion based QS-inhibitory (DDBQSI) assays with the most frequently used QS-signal molecule producing and reporter strains, and to establish standard operating procedures (SOPs) to optimize reproducibility of these assays.

2. Materials and methods

2.1. Culture media

- Mueller-Hinton broth (MH-B) and Mueller-Hinton agar (MH-A) (Bio-Rad Hungary Ltd., Budapest, Hungary)
- Nutrient broth (NB) and Nutrient agar (NA) (Bio-Rad Hungary Ltd., Budapest, Hungary)
- Luria-Bertani broth (LB-B) and Luria-Bertani agar (LB-A) Bio-Rad Hungary Ltd., Budapest, Hungary)
- Modified Luria-Bertani broth (LB*-B) and agar (LB*-A) (which were prepared in-house, containing 8.0 g tryptone, 5.0 g yeast extract, 5.0 g NaCl, 2.0 g glucose, 1.0 g K_2HPO_4, 0.2 g $MgSO_4$ x $7H_2O$, 10 mL 3% $CaCl_2$ stock solution, 5 mL Fe-EDTA stock solution, 1 mL microelement stock solution and 12.0 g of bacteriological agar in case of the solid medium, per 1 L of media; pH was adjusted to 7.0-7.2)

2.2. Bacterial strains

The following bacterial strains were used during our experiments:

- *Chromobacterium violaceum* wt85 [36]
  Taxomony: Gram-negative, facultative anaerobic rod, member of the *Neisseriales* order
  Function: wild-type strain (control strain), characterized by the AHL signal molecule-mediated production of the purple violacein pigment, capable of endogenous QS-signal molecule-production (N-hexanoyl-L-HSL)
- *C. violaceum* CV026 [36]
  axomony: Gram-negative, facultative anaerobic rod, member of the *Neisseriales* order

Function: Tn5 transposase-mutant, AHL-signal molecule indicator strain (produces purple violacein pigment in the presence of AHLs), which is incapable of endogenous QS-signal molecule-production, but useful in the detection of external stimuli

- *Enterobacter cloacae* (clinical isolate no. 31298, isolated from a wound sample) [37]
  Taxomony: Gram-negative, facultative anaerobic rod, member of the *Enterobacterales* order
  Function: AHL-producing-strain (used with *C. violaceum* CV026)
- *Sphingomonas paucimobilis* Ezf 10-17 (isolated from a tumor of the "Ezertűfű" variety of the common grape vine [*Vitis vinifera*]) [36]
  Taxomony: Gram-negative, strict aerobic rod, member of the *Sphingomonadales* order
  Function: AHL-producing-strain (used with *C. violaceum* CV026)
- *Novosphingobium* spp. Rr 2-17 (isolated from a tumor of the "Rajnai rizling" variety of the common grape vine [*Vitis vinifera*]) [36]
  Taxonomy: Gram-negative, facultative anaerobic rod, member of the member of the *Sphingomonadales* order
  Function: AHL-producing-strain (used with *C. violaceum* CV026)
- *Serratia marcescens* AS-1 (Szeged Microbiological Culture Collection) [39]
  Taxonomy: Gram-negative, facultative anaerobic rod, member of the *Enterobacterales* order
  Function: characterized by the production AHL signal molecule-mediated production of the orange-red pigment prodigiosin (2-methyl-3-pentyl-6-methoxyprodigiosin), capable of endogenous QS-signal molecule-production (N-hexanoyl-L-HSL)
- *A. tumefaciens* NTL4(pCF218)(pCF372) (isolated from a tumor of a wild cherry tree [*Prunus avium*]) [36,38]
  Taxonomy: Gram-negative, facultative anaerobic rod, member of the *Rhizobiales* order
  Function: characterized by the expression of β-galactosidase in the presence of a wide range of AHL signals, which may be detected in the presence of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) in the medium, resulting in a color change
- *A. tumefaciens C58* [36,38]
  Taxonomy: Gram-negative, facultative anaerobic rod, member of the *Rhizobiales* order
  Function: AHL-producing-strain (used with *Agrobacterium tumefaciens* NTL4(pCF218)(pCF372))

The bacterial strains for our experiments were kindly provided by Dr. Ernő Szegedi (Institute of Viticulture and Enology, National Agricultural Research Center). The bacterial strains were maintained on Luria-Bertani (LB) agar for shorter time periods (<1 month), while for longer periods, the strains were kept in a -80°C freezer, in a 1:4 mixture of 85% glycerol and liquid Luria-Bertani media. For the maintenance purposes of *C. violaceum* CV026 and *A. tumefaciens* NTL4(pCF218)(pCF372), media were also supplemented with kanamycin and carbenicillin, respectively [36,38].

2.3. Chemicals

Bacteriological agar (Bio-Rad Hungary Ltd.; Budapest, Hungary), tryptone (Thermo Fischer Scientific; Waltham, US), yeast extract (Thermo Fischer Scientific; Waltham, US), D-glucose (Sigma-Aldich; Budapest, Hungary), kanamycin (Sigma-Aldich; Budapest, Hungary), carbenicillin (Sigma-Aldich; Budapest, Hungary), NaCl (Sigma-Aldich; Budapest, Hungary), K_2HPO_4 (Sigma-Aldich; Budapest, Hungary), KH_2PO_4 (Sigma-Aldich; Budapest, Hungary), $MgSO_4x7H_20$ (Sigma-Aldich; Budapest, Hungary), $CaCl_2x2H_2O$ (Sigma-Aldich; Budapest, Hungary), $FeSO_4x7H_20$ (Sigma-Aldich; Budapest, Hungary), Na_2EDTA (Sigma-Aldich; Budapest, Hungary), $MnSO_4x7H_2O$ (Sigma-Aldich; Budapest, Hungary), $ZnSO_4x7H_2O$ (Sigma-Aldich; Budapest, Hungary), $Na_2MoO_4x2H_2O$ (Sigma-Aldich; Budapest, Hungary), $CoCl_2x6H_2O$ (Sigma-Aldich; Budapest, Hungary), dimethyl-sulfoxide (DMSO; Sigma-Aldich; Budapest, Hungary), acridine orange (Sigma-Aldich; Budapest, Hungary) and phosphate buffered saline (PBS; Sigma-Aldich; Budapest, Hungary). During the preparation of the modified Luria-Bertani broth (LB*-B) and agar (LB*-A), the following stock solutions were used: 5% Fe-EDTA stock solution, 3% $CaCl_2$ stock solution and a microelement stock solution (containing 1.0 g $MnSO_4x7H_2O$, 0.5 g $ZnSO_4x7H_2O$, 25 mg $Na_2MoO_4x2H_2O$ and 2.5 mg $CoCl_2x6H_2O$ per 100 mL). The stock solutions were aliquoted in 50 mL centrifuge tubes and kept at -20°C.

2.4. Evaluation of growth characteristics and pigment production of relevant bacterial strains

To identify the ideal experimental conditions, growth characteristics of the bacterial strains used were determined in Nutrient broth (NB), Mueller-Hinton (MH-B) and Luria-Bertani (LB-B) broths,

in addition to Nutrient agar (NA), Mueller-Hinton (MH-A) agar and Luria-Bertani agar (LB-A). In the assays, liquid and solid media were inoculated with the same primary culture for each bacterial strain using a calibrated loop (10 μl). The optical density of the liquid media (OD_{580}, using a photometer) and the number of colonies as well as the degree of pigment production were observed. Growth properties were studied at four different temperatures of incubation: 0°C (refrigerator), 10°C (cooled room with controlled temperature), 25°C (room temperature) and 37°C (incubator). The cultures were measured/read after 12, 24, 48 and 72 hours of incubation. The results of the experiments were from at least three independent experiments. Based on literature data, our study was later complemented with a modified Luria-Bertani (LB*) medium, which was compared to the classical LB medium [42] (see *2.1. Culture media*).

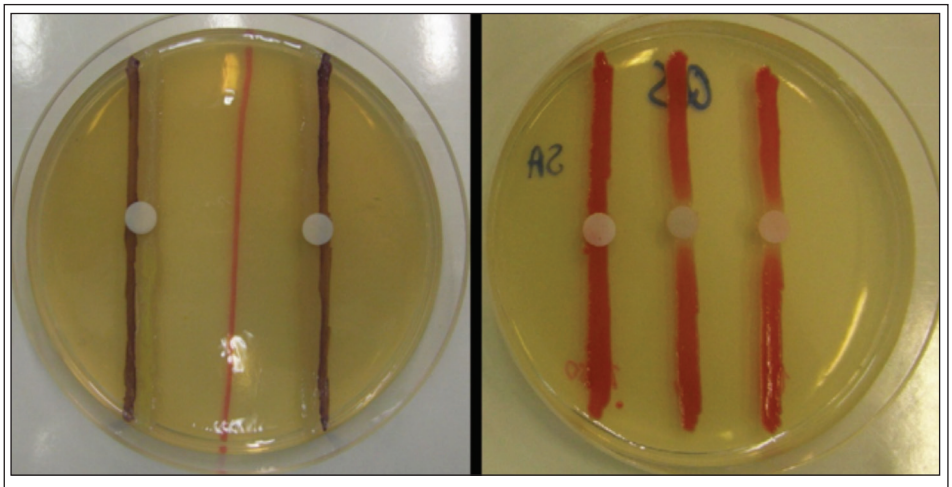

Figure 2 *Disk diffusion quorum-sensing inhibitory assay using C. violaceum CV026 and E. cloacae 31298 (left) and S. marcescens AS-1 (right)*

2.5. Disk diffusion quorum-sensing inhibitory assay

Quorum sensing inhibitory activity was monitored by the disk diffusion method. During the assay, cultures of OD_{580}~0.5 overnight bacteria grown in LB*-B broth were inoculated directly onto LB*-A agar surface. Filter paper disks (7.0 mm in diameter, Whatmann 3MM), were impregnated with 10 μL of acridine orange (AO; used as a positive control; 25.0 mg/mL in phosphate buffered saline) or DMSO (used as a negative control, 2 V/V%) [37]. The disks were placed on the surface of LB*-A agar surface between the parallel inoculations of sensor (*C. violaceum* CV026) and AHL-producer (*S. paucimobilis* Ezf 10-17, *Novosphingobium* spp. Rr 2-17 and *E. cloacae* 31298) strains; the exception was *S. marcescens* AS-1 (capable of producing prodigiosin from endogenous AHL-signals), where disks were placed on the center of the inoculated line (*Figure 2*) [36-39]. To quantify the QS inhibitory effect, the diameter of the QS-inhibition zones (i.e., the culture of discolored but intact bacteria) around the disks was measured using a ruler, after 12, 24, 48 and 72 hours of incubation [36-39]. The results of the studies are derived from the average of at least three independent experiments. The *A. tumefaciens* NTL4(pCF218)(pCF372) and *A. tumefaciens* C58 indicator-AHL-producer pair was not included in this experiment, as the presence of X-gal is required in the media for the colour change to occur.

3. Results and discussion

3.1. Growth characteristics of bacterial strains

There were no relevant differences detected in the growth rate of bacterial strains between the different liquid broths (NB, MH, LB). The growth of bacterial strains was inhibited at low temperatures (0 and 10 ° C) resulting in OD_{580} values of 0-0.05, 0.05-0.1 and 0.1-0.2 for 12, 24, 48 and 72 hours of incubation, respectively, which was inadequate to perform further experiments. There was no difference in bacterial growth between 25°C and 37°C incubations (resulting in OD_{580} values of 0.4-0.5 after 12 hours (i.e. overnight), 0.8-1 after 24 hours, and >1 after 48 hours), except in the case of *C. violaceum* wt85 and *C. violaceum* CV026, where higher reads were observed at 37°C, but in both cases, the OD of the bacterial cultures was appropriate for performing further experiments. The use of 48 hour- and 72 hour-cultures is not recommended, due to the accumulation of dead bacterial cells and autolysis, a consequence of the depletion of nutrients in the culture media (in fact, the OD_{580} values after 72 hours showed decreasing tendencies), which may lead to distorted results in the experiments later on. Similarly, there were no relevant differences detected in the growth rate of

bacterial strains between the solid agar media (NB, MH-B and LB-B). It should be highlighted, that in case of *S. marcescens*, the temperature had a pronounced effect on pigment production in both liquid and solid media (pigment production ceased at 37°C, this effect was not observed for *C. violaceum* wt85). For this reason, 25°C was set as the reference temperature for the additional experiments.

Based on previous reports, it was found that the concentration of several metal ions in the environment has a pronounced effect of quorum sensing in bacteria [37]. After a thorough literature survey, an additional medium was included in our optimization studies, namely the modified LB (or LB*) broth and solid media, which is supplemented by additional nutrients and a microelement solution (containing various metal ions) [42]. The tested strains showed no relevant differences in the growth characteristics in LB-B and LB*-B broths in the same experimental setup previously described. However, during the comparison of LB-A and LB*-A solid media, it was evident that colony formation (number and size of bacterial colonies) and pigmentation of the colonies occurred more rapidly, therefore, the growth properties of the relevant strains were further characterized on this media (*Table I*). During the bacterial growth experiments on the LB*-A solid media, it was observed that bacterial colonies' growth and pigment production on LB * agar were stable after 48 hours when incubated at 25°C. In addition, if the read-

Table I *Growth characteristics of tested QS-strains on LB*-A media at room temperature (25 °C)*

	Optical density (OD_{580}) of bacterial inoculum used				
After 24 hours	0.1	0.3	0.5	0.7	1.0
***Chromobacterium violaceum* CV026**	ø	ø /+	+	+	++
***Chromobacterium violaceum* wt85**	ø	ø/+	+	+	++ (!)
***Sphingomonas paucimobilis* Ezf 10-17**	ø/+	ø/+	+	++	++
***Novosphingobium spp.* Rr 2-17**	ø	ø	ø	+	+
***Serratia marcescens* AS-1**	++	++	+++	+++	+++
***Enterobacter cloacae* 31298**	++	++	++	+++	+++
***Agrobacterium tumefaciens* NTL4**	-/+	-/+	+	++	++
***Agrobacterium tumefaciens* C58**	-/+	-/+	+	++	++
After 48 hours	0.1	0.3	0.5	0.7	1
***Chromobacterium violaceum* CV026**	++	+++	+++	+++	+++
***Chromobacterium violaceum* wt85**	+++ (!)	+++ (!)	+++ (!)	+++ (!)	+++ (!)
***Sphingomonas paucimobilis* Ezf 10-17**	++	++	+++	+++	+++
***Novosphingobium spp.* Rr 2-17**	++	++	++	++	+++
***Serratia marcescens* AS-1**	+++ (!)	+++ (!)	++++ (!)	++++ (!)	++++ (!)
***Enterobacter cloacae* 31298**	+++	+++	+++	+++	+++
***Agrobacterium tumefaciens* NTL4**	+++	+++	+++	+++	+++
***Agrobacterium tumefaciens* C58**	+++	+++	+++	+++	+++
After 72 hours	0.1	0.3	0.5	0.7	1
***Chromobacterium violaceum* CV026**	+++	+++	+++	+++	+++
***Chromobacterium violaceum* wt85**	+++ (!)	+++ (!)	+++ (!)	+++ (!)	+++ (!)
***Sphingomonas paucimobilis* Ezf 10-17**	+++	+++	+++	+++	+++
***Novosphingobium spp.* Rr 2-17**	++	+++	+++	++++	++++
***Serratia marcescens* AS-1**	+++ (!)	+++ (!)	++++ (!)	++++ (!)	++++ (!)
***Enterobacter cloacae* 31298**	+++	+++	++++	++++	++++
***Agrobacterium tumefaciens* NTL4**	+++	+++	++++	++++	++++
***Agrobacterium tumefaciens* C58**	+++	+++	++++	++++	++++

Legend: ø: no growth, +: weak bacterial growth, ++: moderate bacterial growth, +++: adequate bacterial growth, ++++: strong bacterial growth (!): pigment production

ing of the plates occurred after 48 hours, the colony growth and pigment production has shown to be independent from the optical density of the initial inoculum in the range OD_{580}≥0.5, while this number was OD_{580}≥0.1 if the reading occurred after 72 hours (*Table I*).

3.2. Disk-diffusion quorum-sensing inhibitory assay

The results of the optimization experiments with the positive control acridine orange (AO) are presented in *Table II*, where the quorum-sensing inhibition zones are shown for the parallel inoculations between QS-sensor strain *C. violaceum* and the AHL-producer strains, and for *S. marcescens* AS-1, respectively (*Figure 3*). No quantifiable QS-inhibition zone (i.e. loss of purple violacein pigmentation) was detected in case of the CV026-AHL-producers after 12 hours, at least 24 hours were needed for the discoloration to develop, except for the *S. marcescens* AS-1, where minor inhibition was present. Based on our results, the inhibition zone was still subject to change at the 24 hour-reading of plates, however, the results after 48 hours may be considered to be final, additional incubation and observation did not change the results. According to the data presented, the *Serratia* model system was the most sensitive for the QS-inhibitory activity of AO. DMSO was used as a negative control, no measureable QS-inhibition zone was detected.

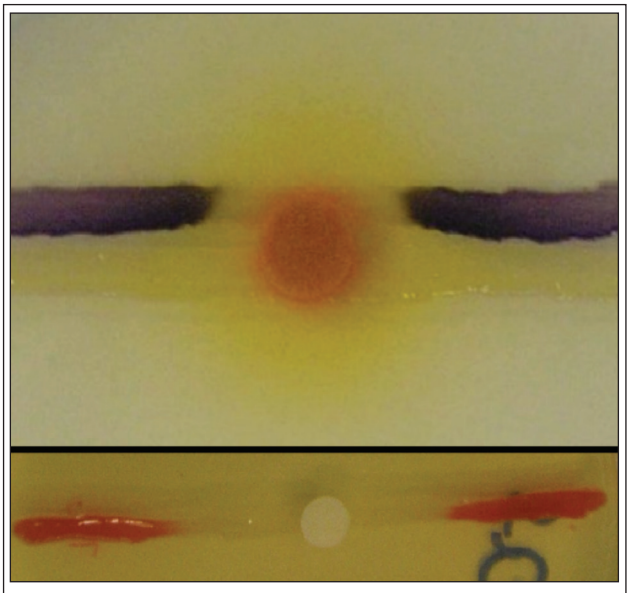

Figure 3 *Quorum sensing-inhibition zones using C. violaceum CV026 and E. cloacae 31298 (top) and S. marcescens AS-1 (bottom) after 48 hours of incubation*

4. Conclusions

The emergence of multidrug resistance in bacterial infections significantly hinders the appropriate therapy of patients, and with the current disinterest of pharmaceutical companies to develop new antibiotics, alternative approaches should be considered for the therapy of these infections. Quorum sensing is a form of bacterial cell-cell communication, whereby these microorganisms use diffusible signal molecules as proxy to detect the surrounding cell density and produce metabolically costly products when the sufficient biomass has been reached. Inhibitors of quorum sensing may be potent modulators of bacterial virulence, eliminating their pathogenic potential, without killing them (therefore the selection pressure would be lower), however, the development and screening for the QS-activity of these compounds is not well-established. A standard operating procedure (SOP) is a designated set of step-by-step instructions compiled by relevant (qualified) individuals or an organization to help carry out various operations, aiming to increase precision and efficiency. The aim of our study was to characterize the appropriate conditions for the disk diffusion-based QS-inhibition assay, consisting of QS-signal sensor and AHL-producer strains. Based on our results, the ideal experimental setting includes a modified Luria-Bertani medium (complemented with nutrients and microelements), incubation at room temperature (25°C) for 48 hours before reading of the results, where the density of the starting

Table II *Quorum-sensing inhibitory activity of acridine orange (OA) in various model systems, corresponding to different plate-reading times*

	Quorum-sensing inhibition zone diameter (mm±SD)			
Bacterial model system	12 hours	24 hours	**48 hours**	72 hours
***C. violaceum* CV026 + *E. cloacae* 31298**	ø	13 ± 2.2	**14 ± 1.2**	14 ± 1.2
***C. violaceum* CV026 + *S. paucimobilis* Ezf 10-17**	ø	14 ± 1.6	**16 ± 0.9**	16 ± 0.9
***Novosphingobium* spp. Rr 2-17**	ø	11 ± 2.0	**13 ± 1.0**	13 ± 1.0
***Serratia marcescens* AS-1**	6 ± 2.3	17 ± 1.5	**19 ± 0.8**	19 ± 0.8

inocula has less influence of the results of the assay. Adherence to the abovementioned criteria may aid in improving the reproducibility, comparability and reliability of results obtained by this method.

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6. Competing interests

The authors declare no conflict of interest, monetary or otherwise.

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Patient adherence and factors influencing quality of life in the case of osteoarthritic patients

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Abstract
Introduction: *135 million humans are affected by osteoarthritis worldwide, which is going to be doubled by 2020. Current treatment options are limited and complex, requiring the active participation of the patients in order to reach the optimal therapeutic outcome. To date, there is no study available measuring the adherence and quality of life of Hungarian arthritic patients.*
Aims: *Evaluation of risk factors affecting quality of life and adherence in the case of Hungarian patients, in order to identify the potential intervention points.*
Methods: *Hospitalized patients' final reports were analyzed retrospectively (gender, age, BMI, therapy, co-morbidities).*
Results: *400 cases were evaluated (females 69%) with an average age of 72 years. 80% of the patients had abnormal BMI. Non-pharmacological treatments ensured the longest asymptomatic period, medication histories showed polypharmacy. Co-morbidities were observed in almost every case.*
Conclusion: *Patient centeredness is necessary, based on multidisciplinary healthcare team to support the expected quality of life.*

Keywords: *osteoarthritis, adherence, quality of life.*

1. Introduction

In chronic conditions, the World Health Organization (WHO) considers it essential to maximize the patients' willingness to co-operate with therapies; and it classified the factors influencing adherence into 5 dimensions. The study was made in 2003 and it is still regarded as a standard document by researchers studying patient adherence. With regards to the ever aging societies, like Hungary, needs to highlight the prevalence and the incidence of the chronic disorders. [1]. Among chronic conditions, diseases affecting the musculoskeletal system are on the rise; besides the basic disease, these cause several other diseases, loss of quality of life, loss of working capacity on the labour market, etc., and thus they put an increasing burden on the patients concerned and on the society both regarding quality of life and in economic terms.

The importance and the severity of the issue were recognized worldwide and the 'Decade of Bones and Joints' was proclaimed in 2000, which was extended until 2020 due to its success, and several other European organizations have also taken steps to focus attention on the disease [2-4]. Hungary was the first to join the program on state level and achieved major success in many areas [5, 6]. In spite of all these efforts, very few Hungarian studies have been made and published on OA (erosion of articular cartilage).

Arthrosis is the leading cause of disability and pain all over the world and was ranked as the 6th most common cause of disability in 2003, estimated to be the 4th in rank in 2020 [7-9]. In Hungary, the number of patients affected can only be estimated; during the European Health Interview Survey of 2014, 17% of the people asked were found to have arthrosis-related joint pain, which can mean the involvement of about ~ 1,600,000 people nationwide [10].

The measurement of the quality of life and adherence of patients suffering from OA is complicated by several factors, which are the following:

– The disease is usually diagnosed when it is already in an advanced stage, i.e. when there are severe symptoms. The therapeutic options are

List of abbreviations: *Osteoarthrosis – OA; World Health Organization – WHO; National Institute for Health and Care Excellence – NICE; Non-Steroidal Anti-Inflammatory Drug – NSAID*

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limited; what can be achieved is mainly the alleviation of the symptoms for a longer or shorter period of time [11-13].
- As opposed to other musculoskeletal disorders, no well-proved disease-modifying active ingredient is available [14, 15].
- Due to the complex nature of the disease, the patient's active and conscious cooperation with the healthcare professionals involved in the treatment is essential.

The therapy is described in the NICE guideline [16], which divides the tasks into three separate parts. The first and foremost is the education of patients during treatment, both by doctors and pharmacists, which includes the promotion of a healthy lifestyle, the incorporation of sports into everyday life, i.e. health-conscious behavior; and also adopting the so-called Mediterranean diet [17]. All these reduce the risk of the most important risk factor, obesity, as well as the development or worsening of cardiovascular diseases, diabetes mellitus and mental illnesses, which are also risk factors in the incidence of OA.

Another element of therapy is the use of non-pharmacological therapies, which include electrical impulse therapies, manual therapies and balneological therapies. Currently, these provide the longest asymptomatic period. Paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs), usually applied topically then orally, are the drugs of first choice in medicated therapies. If they fail to be effective, the administration of the opioid analgesic tramadol is recommended. Most of the active ingredients mentioned in the first group are also available in drugs without a prescription, so the extent of their use cannot be measured in this disease; it is well-known that their excessive and combined use generates several adverse effects and causes additional burdens.

The protocol does not recommend the use of chondroprotective drugs, although patients often expect these "miracle drugs" to rebuild cartilage. Commercially available products are available to users through a number of distribution chains, frequently bypassing healthcare professionals; this can result in uncontrolled use if communication between the parties is inadequate during patient care.

The rational use of the great number of over-the-counter drugs and chondroprotective preparations can be controlled only by the pharmacist, who can provide information to the family doctor and to the specialist, and can also recommend the patient to visit the doctor.

On the other hand, physicians and physiotherapists can inform the pharmacist about the patients' medical history, who can then dispense the medication with this knowledge.

In the light of all these facts, it can be hypothe-

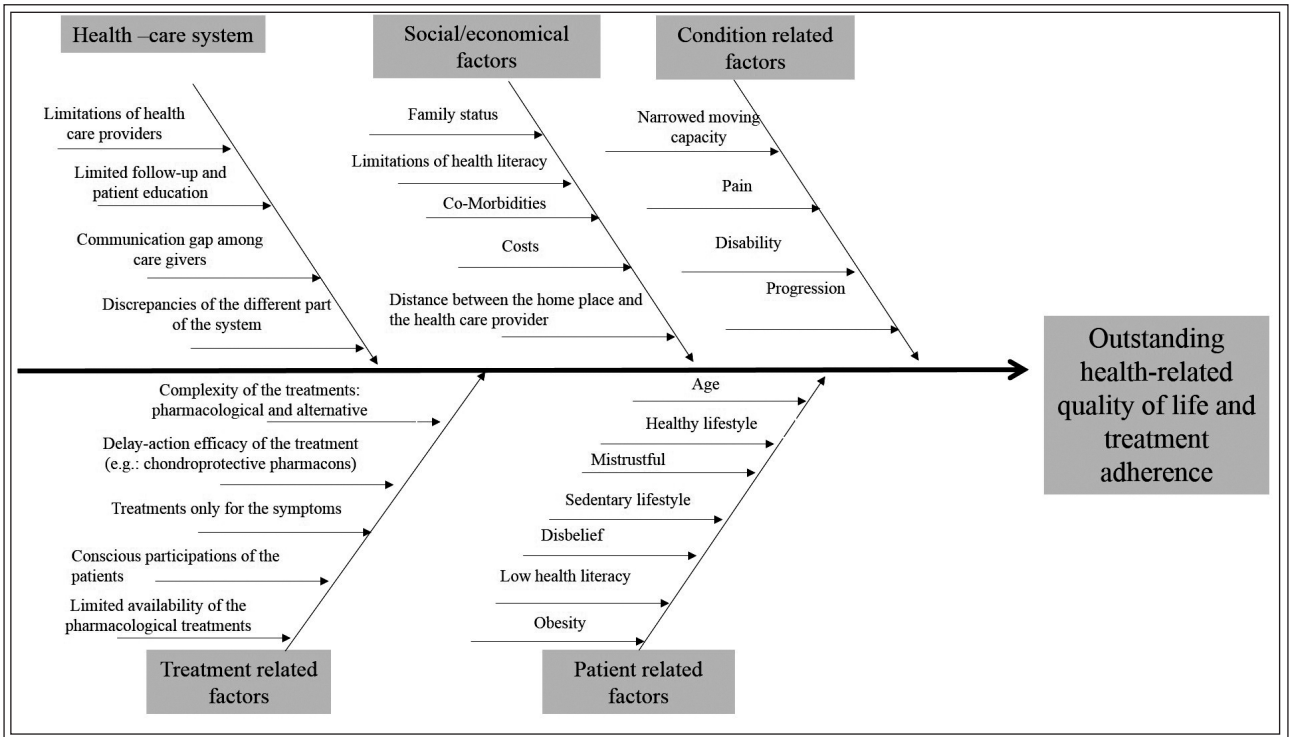


Figure 1 *Division of the population in question by age and gender*

sized that adherence can be improved if OA patients' burdens affecting therapeutic cooperation are explored. With the improvement of adherence, patient satisfaction and cooperation with therapy improves, which in turn has a favourable influence on specific parameters of quality of life, resulting in improvements at individual, family and social levels. Given the complexity of the therapy, this can be achieved only through the collaboration of the healthcare professionals participating in the treatment, which will improve therapeutic efficiency. Therefore, the aim of this survey is to explore the most common causes in order to identify potential intervention points based on the 5 dimensions of adherence of the WHO. The causal (Ishikawa) diagram (*Figure 1*) made on the basis of literature research illustrates the factors that influence the quality of life and cooperation of arthritic patients, which, at the same time, can also be the most important intervention points for improving the therapeutic outcome.

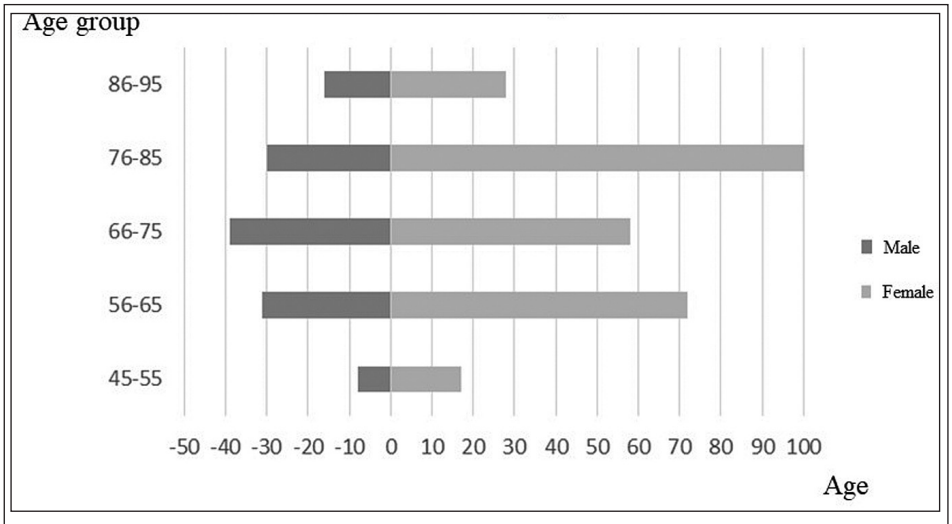


Figure 2 *Cause and effects relationships between factors influencing osteoarthrosis*

2. Methods

As the first step, the affected population was assessed retrospectively, in the course of which the data of patients treated by the Bács-Kiskun County Teaching Hospital of the University of Szeged, the Musculoskeletal Rehabilitation Unit of the Establishment in Kiskunfélegyháza was processed. During the data collection period we randomly selected five anonymised patient discharge reports

Documents from the period of 2007-2013 were selected for the study; the inclusion criterion was the diagnosis of lower limb OA.

Assessment aspects:

- gender
- age
- body mass index (BMI)
- residence,
- average of nursing days,
- non-pharmacological therapies received during hospitalization, medicines received,
- existing co-morbidities.

Statistical evaluation was performed with Microsoft Excel 2013 and Version 23 of the SPSS program package.

The study was conducted with the permission of the Scientific and Research Ethics Committee, file number: 24950-3/2016/EKU.

Table I *Descriptive statistics regarding to age*

Aspects		Values
Number of patients (N)	Valid	400
	Missing	0
Mean		72.10
Median		74.00
Std. Deviation		10.81
Minimum		45.00
Maximum		93.00

Table interpretation:

- *N: sample size*
- *Valid: Actual number of sample size*
- *Missing: Missing number of sample*
- *Mean: Average age based on the evaulated sample*
- *Median: Median of the age based on the evaulated sample*
- *Standard Deviation (Std. Deviation): Dispersion of the age of the examined sample around the mean age*
- *Minimum: The lowest age in the sample tested*
- *Maximum: The highest age in the sample tested*

3. Results

3.1. Gender and age of patients

A total of 400 final reports were evaluated during the study period. The majority of cases (69%) were female with an average age of 72 years (*Table I*). It can be seen that the majority of patients were in the age group 76-85 for women and 66-75 for men (*Figure 2*). This result is not surprising as the number of patients who visit a healthcare institution

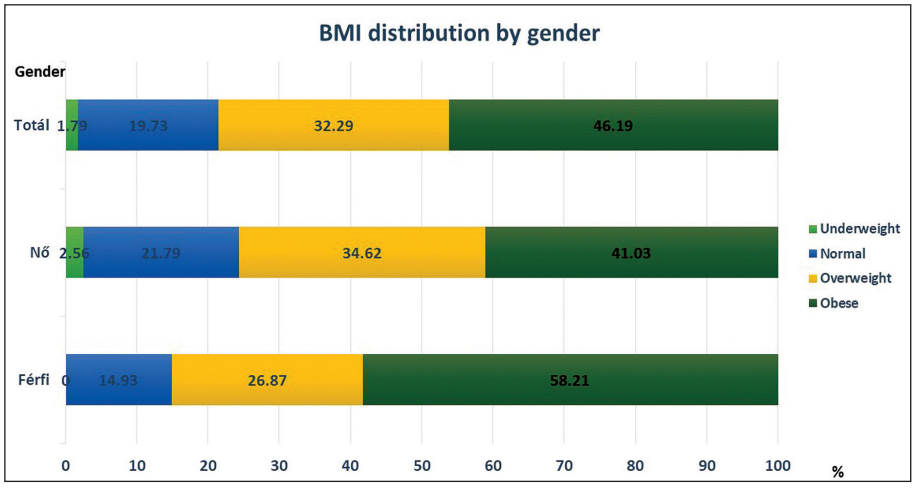


Figure 3 *Division of the population in question by Body Mass Index BMI categories in accordance with WHO classification : <18.5 underweight, 18.51 – 24.9 normal, 25.0 -29.99 overweight, >30.0 obese*

with complaints increases dramatically over the age of 60. As regards age analysis, it should be noted that 128 patients (32%) were under 65 years of age, i.e. in active working age. Considering that the average of nursing days for the sample examined was 18.6 days, this means that the affected persons were unable to work and to perform their duties minimum for this period. There was no significant relationship (p = 0.075, NS) between gender and age at significance level 0.05

3.2. Relationship of body mass index (BMI) with gender and age

The most important risk factor for arthrosis is overweight. Inadequate body mass puts enormous strain on the joints and cartilage, which are eroded continuously during daily activities; and this can be further aggravated by the lack of sports activities in the daily routine.

From among the 400 cases examined, the data necessary for BMI calculation (body weight and height) were available in 224 cases. The results are summarized in *Figure 3* ~ 46% of the patients were found to be in the most severe "obese" category; overall, ~ 80% of the cases deviated from the "normal" body weight category (BMI categories, based on the classification of the WHO). There was no significant difference between men and women (p = 0.148). Such a serious weight problem not only enhances the development and progression of musculoskeletal disorders but also forms the basis of many other chronic conditions, which increase the likelihood and frequency of hospitalization as well as the number and quantity of medications taken.

3.3. Hungarian data related to the recommendations of the NICE disease-specific guideline

Today the treatment of arthrosis is primarily limited to relieving symptoms; the reason for this is that although the development of therapies for the modification of the course of the disease is researched extensively Currently there is no active ingredient available with an adequate level of evidence and the treatment related risk is still higher than the therapeutic benefit

For the sake of completeness, it should be noted that patient education recommended in the first place is going to be the subject of the following study; no data regarding it were available in the current retrospective survey.

3.4. Non-pharmacological therapy

The guideline identifies non-pharmacological therapeutic options as the level following patient education. These treatments can be received in the rehabilitation centers operating in healthcare institutions or in qualified spas, with a specialist's referral. As the studied population underwent musculoskeletal rehabilitation treatment, our study also included the evaluation of non-pharmacological treatments received during hospitalization. The guideline groups therapies according to their focus on muscle strengthening, gymnastic exercises or the use of electrical impulse. We grouped the survey data according to this, and if they did not specifically belong to any of the groups, they were classified into the so-called "other" category. The results are shown in *Figure 4*.

Gymnastic exercises included individual and group exercises performed on land and in water, with or without help (striped marking). Mobilization, weight bath and medicinal bath were grouped into the category of muscle strengthening (black marking); while treatments based on electrical impulse included Tangentor, galvanic treatment, iontophoresis, TENS and the use of BEMER mattress (checkered marking). Ultrasound and mud pack were classified in the "other" category (grey marking).

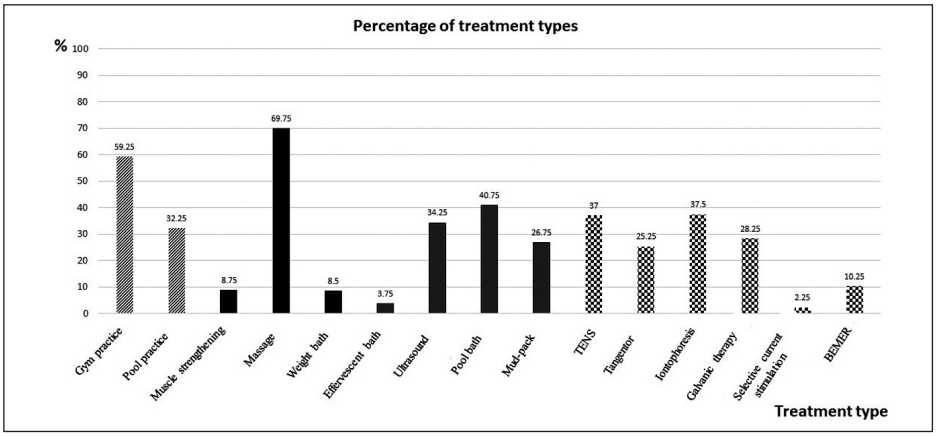
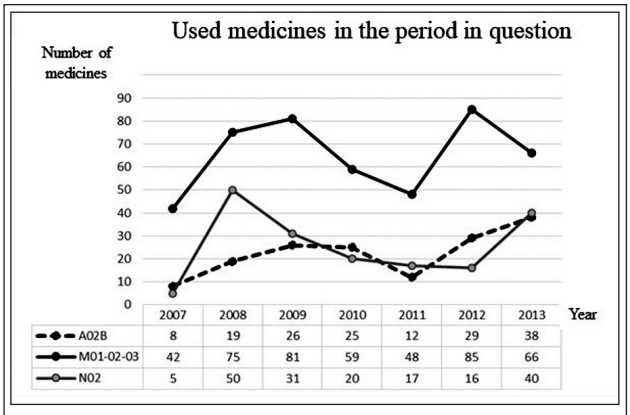


Figure 4 *Percentage distribution of non-pharmacological therapies during the study period*



	2007	2008	2009	2010	2011	2012	2013
A02B	8	19	26	25	12	29	38
M01-02-03	42	75	81	59	48	85	66
N02	5	50	31	20	17	16	40

Figure 5 *Frequency of the pharmacons according to relevance with the disorder in question*
Distribution of the used pharmacons according to ATC categories
- *A02B: Drugs for peptic ulcer and gastro-oesophageal reflux disease (GORD)*
- *M01-02-03 Anti-inflammatory and antirheumatic products, Topical products for joint and muscular pain, Muscle relaxants*
- *N02: Analgesics*

Regarding to the Figure is demonstrated the number of medicine boxes diveded by years. The pharmacons were counted based on the patients documentation. The mentioned ATC categories (A02B, M01-02-03, N02) were selected in accordance with NICE guideline therapeutic suggestion.

Non-pharmacological therapies were very diverse, all the options in modern medicine were available for the patients. The percentage distribution of the treatments shows that the rates of gymnastic exercises and massage were the highest. Patients received these in ~ 60% and ~ 70% of the cases. When discharged, the patients were recommended to continue gymnastic exercises at home on a daily basis; the nature of the exercises was such that after training the patients could perform them on their own, without continuous expert supervision. Needless to say, their regular performance requires a high degree of patient adherence.

3.5. Evaluation of pharmacological therapy

As already mentioned in the introduction, the NICE guideline recommends the use of paracetamol and the topical application of non-steroidal anti-inflammatory drugs (NSAIDs) as first-line agents; followed by oral NSAIDs as the next step, and finally weak opiates (tramadol and its derivatives); the latter is frequently the first-line therapy for the elderly. Given that paracetamol and the vast majority of NSAID formulations are available without a medical prescription and the diagnosis is not established, the proportion of formulations purchased specifically for musculoskeletal complaints cannot be determined without a special questionnaire survey. Furthermore, the evaluation is limited by the fact that this active ingredient group includes hundreds of active ingredients and there is no clear recommendation for a particular one. Selective inhibitors for cyclooxygenase type 2 enzyme are mostly prescription-only preparations and can thus be monitored. It must be mentioned that their excessive or unjustified use may result in cardiovascular complaints.

The active ingredient diclofenac, which is not selective for the enzyme type, is also the active ingredient in a great number of preparations for musculoskeletal complaints; in this case, many non-prescription drugs are available, and their use for this purpose cannot be assessed without a special survey. It should be noted here that the prolonged and combined use of non-selective NSAIDs may result in the development of severe gastrointestinal adverse effects or in the exacerbation of the existing symptoms; patient education is essential in this stage as well.

Based on the final discharge reports, , a total of 3,313 drugs were administered to 400 patients, which means an average of ~ 8 drugs per patient. Recorded NSAIDs, formulations containing paracetamol and tramadol were included in the evaluation; and also proton pump inhibitors, which the recommendation strongly recommends

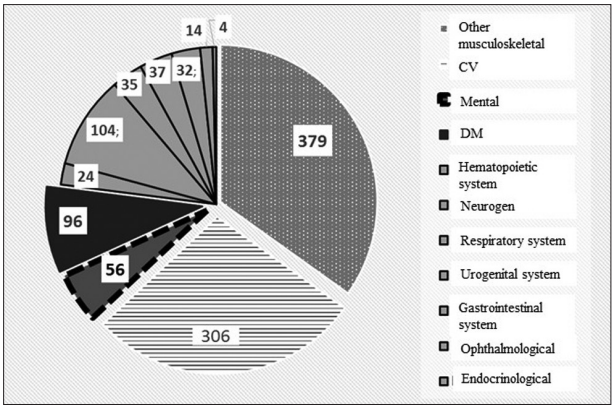


Figure 6 *Incidence of co-morbidities during the period studied*

for use with NSAIDs. The drugs used were analyzed with the consideration of the ATC codes of the active ingredients, the results are summarized in *Figure 5*.

It is noteworthy that first-line paracetamol was mentioned only in 11 cases, while tramadol and its derivatives were taken in 168 cases (group NO2), which had been expected considering the age distribution (grey line). Another important finding is that despite taking a great amount of non-steroids, the number of gastric protectors is negligible (NSAID – black line, PPI – proton pump inhibitors – broken line). The curves of non-steroids and PPIs run essentially parallel to each other in the period studied, which suggests that in some cases PPI was used while taking NSAIDs, but generally only in one third of the cases.

3.6. Existing co-morbidities

Existing co-morbidities were also recorded in the patients' final reports and their evaluation is shown in *Figure 6*. According to the data, the diagnosis contained "other musculoskeletal complaint" almost without exception, which means 379 cases (grey dots). Some cardiovascular disease occurred in 70-80% of the cases in the test sample (black stripes). DM was also present consistently each year with an incidence of 20-30% (black marking), and the basic disease was accompanied by mental/psychological problems in many cases (broken lines).

4. Discussion

In conclusion, as a result of aging societies, arthrosis will result in an increasing number of patients worldwide, thus in Hungary as well. The situation may be worsened by low health literacy, the general lack of health-conscious behavior, and unhealthy and sedentary lifestyles, which are common problems in today's societies.

It is a particularly great challenge to make the diagnosis in time as the disease is "silent" for a while; pain and impaired mobility that interfere with daily activities occur only in the advanced stage of the disease. By this time, arthrosis is of such extent that the affected population needs continuous treatment.

The knowledge of the factors leading to the development of arthrosis and the analysis of the available patient documentation are absolutely necessary to establish the Hungarian population's state and, on the basis of the facts, to explore potential intervention points in order to improve the patients' quality of life.

The current survey, based on the international guideline, processes and evaluates data from a representative sample of Hungarian patients, in the groups of the recommended therapies, broken down according to non-pharmacological and pharmacological therapies.

A significant proportion of the Hungarian patient population selected by randomized sampling is in active working age, and the majority of them belong to the overweight or obese categories.

Non-pharmacological rehabilitation treatments are widespread, they are efficient and result in a long-term asymptomatic period; however, the patients discharged from the treatment have to do the exercises recommended by doctors and physiotherapists on their own at home every day in order to maintain the achieved results, so the patients' conscious adherence is essential.

Pharmacological therapy is characterized mainly by the use of over-the-counter preparations, which the patients commonly take in excess and in combination, parallel to their prescription-only counterparts. This phenomenon may induce adverse effects, the development or worsening of co-morbidities. Irrespective of this, co-morbidities affecting the cardiovascular and metabolic systems are more common in arthrosis, which was highlighted in our study.

5. Conclusion

In order to comply with the guideline on patient education, the multidisciplinary collaboration of Hungarian healthcare professionals working in different areas is required. Besides family doctors,

specialists and physiotherapists, pharmacists also have a prominent role due to the phenomena experienced in pharmacological therapy. Pharmacists taking part in the practice, in collaboration with dietitian and physiotherapist colleagues, can ensure mutual and ongoing communication by providing relevant information to the doctor responsible for therapy. In this way, patient care is shared by professionals with appropriate competences, the excessive use of unnecessary drugs taken on the patient's own initiative can be avoided, the monitoring of recommendations for lifestyle and exercises at home can be shared, and adherence can be maintained longer. The simultaneous and combined use of all these, the collaboration of healthcare professionals is the key to successful therapeutic outcome and the resulting improvement in the quality of life in the case of osteoarthrosis as well.

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