ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3819-3834
doi: 10.25083/rbl/28.1/3819.3834

Received for publication, March, 27, 2023
Accepted, April, 20, 2023

Review

Occupational health improvement study on lumbar paraspinal muscles via surface electromyography during several tasks – a review

D ATHIKESAVAN[1,*], M S ALPHIN[1], SACHIN GAURISHANKAR SARATE[2], K RAMRAJI[3], S MOHAMMED ADHIL[2]

[1] Department of Mechanical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, Chennai, Tamil Nadu, India – 603110.
[2] Department of Bio-Medical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, Chennai, Tamil Nadu, India – 603110.
[3] Department of Mechanical Engineering, Mohamed Sathak Engineering College,Kilakarai- 623806, Tamilnadu, India.

Abstract

The lumbar paraspinal muscles are located next to the vertebrae responsible for lower back motion, and they are a set of muscles with distinct designs and complex features. Lower back muscles are represented by the erector spinae (ES) and multifidus (MF) muscles, which are located between the L3 and L4 vertebrae. Back pain from paraspinal muscular disorders causes muscle atrophy, tension, and poor posture. As a result, it is important to monitor muscle fatigue and the cross-sectional area (CSA) of paraspinal muscles using the EMG approach and imaging techniques. The screening of these paraspinal muscles can evidence changes related to low back pain both before and after exercise; it can be used together with CSA of the paraspinal muscles to assess muscle atrophy caused by disc herniation and spinal stenosis as well as postoperatively. This review will aid researchers in gathering information on numerous elements that influence muscle fatigue and determining the usefulness of studying muscle atrophy in connection to disc herniation and spinal disease using various imaging modalities.

Keywords

Lumbar paraspinal muscles, Electromyography, Cross-sectional area (CSA), Erector spinae (ES), Multifidus (MF).

To cite this article: D ATHIKESAVAN. Occupational health improvement study on lumbar paraspinal muscles via surface electromyography during several tasks – a review. *Rom Biotechnol Lett.* 2023; 28(1): 3819-3834 DOI: 10.25083/rbl/28.1/3819.3834

*Corresponding author: D Athikesavan - Department of Mechanical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, Chennai, Tamil Nadu, India – 603110; email: d.athikesavan@gmail.com

Introduction

The paraspinal muscles include: the quadratus lumborum, psoas major, multifidus, erector spinae, interspinal, and intertransversarii. They maintain the mobility and stability of the spine [1]. The mobility of the lower back is governed by muscles of the back, such as the multifidus and erector spinae. The architecture and design of paraspinal muscles are complicated, and they differ from the architecture and design of other muscles that have been widely studied (such as appendicular muscles). Many skeletal attachments and insertions exist in the paraspinal muscles, for example, at various vertebral levels. The erector spinae muscles serve to stabilize the lower back by combining the longissimus and iliocostalis muscles. The longissimus is in the erector spinae's middle section. Both the medial portion of the transverse process and the auxiliary process supply fibers to each of the five lumbar vertebrae. The multifidus is the biggest and most medial back muscle, spanning the lumbosacral junction. Its job is to keep the trunk erect and allow for abduction and rotation[2, 3].

Back pain caused by paraspinal muscular diseases can induce muscle atrophy, muscle strain, and poor posture. With 65–85 percent of the population reporting having lower back pain at some time in their life, it is becoming more and more prevalent [4]. As a result, it is crucial to consider the factors that impact muscular exhaustion in certain muscles, as well as their cross-sectional area (CSA), because fatty infiltration is more likely to occur. Fat and fibrous tissues can occasionally replace atrophied muscle, resulting in a decrease in muscular activation. Such phenomena render assessing muscle's CSA imminent.

The paraspinal muscles can be evaluated quantitatively and qualitatively using MRI, CT, and f-MRI images to measure CSA and determine the presence of fatty infiltration. To reveal the elements that produce muscular fatigue, researchers are analyzing EMG data from people with both acute and chronic lower back pain.

Electrodes inserted in skeletal muscles are used to detect electrical impulses from motor neurons in the central nervous system (CNS). Biomechanics uses these signals to evaluate movements and diagnose medical issues and activation levels. HD-sEMG is a non-invasive technique that covers a small patch of skin with more than two evenly spaced electrodes to measure electrical muscle activity. The ability to capture both temporal and spatial EMG activity with HD-sEMG enables the identification of new muscle properties [5]. In neurogenic diseases and channelopathies, HD-sEMG can reveal pathologic MU alterations [6]. Prior to and following exercise both acute and persistent lower back pain CSA values obtained from imaging modalities are also used to evaluate paraspinal muscle atrophy in the setting of spinal stenosis and disc herniation in people who currently feel or have in the past experienced back pain. Some research studies were inspired by [7], which describes assessment of fat infiltration in lumbar paravertebral muscle for LBP, and how imaging modalities like Dual Energy CT (DECT) were more efficient here. Similarly, many literature studies have been performed by paraspinal muscles monitoring using different approaches and have been discussed throughout this manuscript. Figure 1 shows an MRI of the L3-L4 disc level revealing the default size and location of the paraspinal muscles.

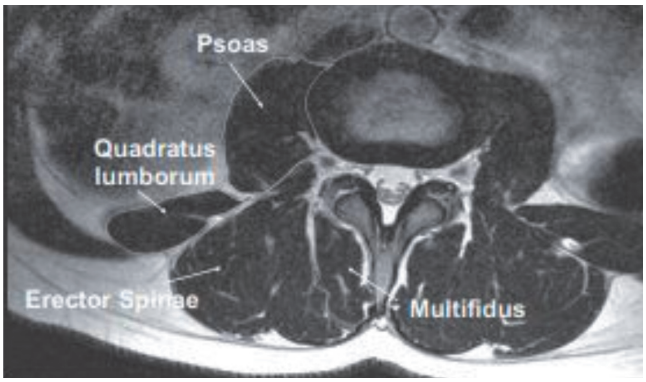


Figure 1. MRI of L3-L4 disc level showing paraspinal muscles around it.

Study Criteria

The present review analyzes the impact on paraspinal muscles during low back pain, during the flexion-relaxation phenomena, and their structural alterations that may be examined using medical images. The flow chart of studies selection according to well-defined criteria is shown in Figure 2.

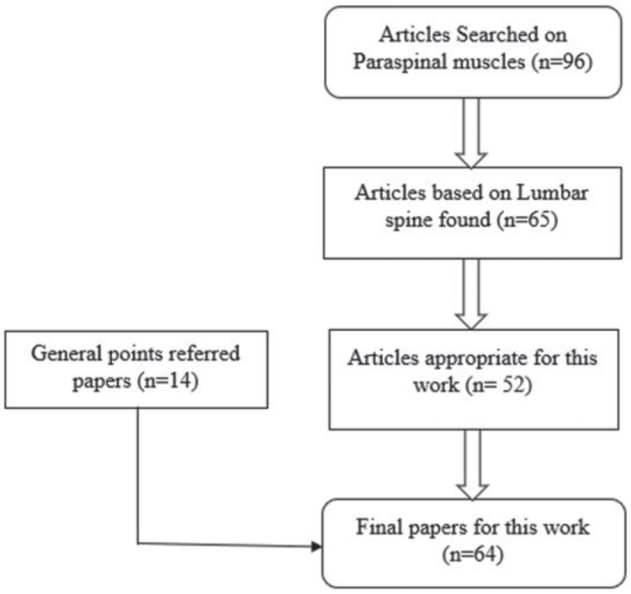


Figure 2. Flow chart of study criteria.

Need of EMG variables in the study

To compare the characteristics of those who experienced low back pain (LBP) with those who did not, as well as to determine the degree of their discomfort, EMG data from lumbar paraspinal muscles were required. Figure 3 and Table 1 describe electrode location from L1 to L5. EMG from these studies also aids in the understanding of FRP (Flexion-Relaxation Phenomenon), providing a thorough examination of lumbar muscle activity [8, 9, 10].

Table 1. Electrode placement in each channel.

Sl.No	Channel Name	Muscle
1	Channel 1	Erector spinae (ES) (left L1/L2 level)
2	Channel 2	Erector spinae (ES) (right L1/L2 level)

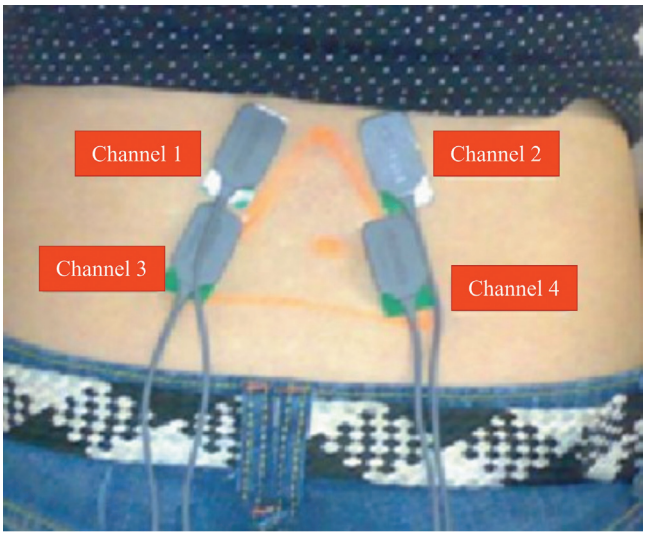


Figure 3. Electrode position at L1 to L5(S. ARJUNAN & al., 2010 [10]).

3	Channel 3	Multifidus (MF) (left L4/L5 level)
4	Channel 4	Multifidus (MF) (right L4/L5 level)

EMG Feature Extraction

The method of feature extraction emphasizes important information by removing noise from the raw signal data. An EMG-based control system must include three types of characteristics to function properly. These are the time, frequency, and time-frequency domains [11, 12].

Time domain

For EMG pattern identification, time-domain features are more typically utilized. This is due to the fact that they are simple and quick to calculate because no transformation is required. The amplitude of the input signals is used to construct time domain characteristics. The generated numbers offer an approximation of waveform amplitude, frequency, and duration within defined bounds [13]. The next subsections give complete descriptions on integrated EMG, mean absolute value, mean absolute value slope, Simple Square integral, EMG variance, root mean square, and waveform length.

(i) Integrated EMG (IEMG)

To know the pre-activity of muscles Integrated EMG (IEMG) is widely used. They are the areas under the curve of rectified EMG signals and they are also the summation of absolute EMG amplitude [14].

$$IEMG = \sum_{i=1}^{N} |x_i| \quad (1)$$

(ii) Mean Absolute Value (MAV)

The intensity of muscular contractions is found and measured using the Mean Absolute Value (MAV) technique. It is calculated as the full-wave rectified EMG signal's moving average [12, 14].

$$MAV = \frac{1}{N}\sum_{i=1}^{N} |x_i| \quad (2)$$

(iii) Mean Absolute Value Slope (MAVS)

To determine the difference in MAVs between neighboring segments, one uses the Mean Absolute Value Slope [14].

$$MAVS_i = MAV_{i+1} - MAV_i \quad (3)$$

(iv) Simple Square Integral (SSI)

The Simple Square Integral (SSI) is used to represent the EMG signal's energy into an useable characteristic [14].

$$SSI = \sum_{i=1}^{N} |x_i{}^2| \quad (4)$$

(v) Variance of EMG (VAR)

The EMG signal's strength is expressed as a usable property via the Variance of EMG (VAR) [12, 14].

$$VAR = \frac{1}{N-1}\sum_{i=1}^{N} x_i^2 \quad (5)$$

(vi) Root Mean Square (RMS)

There is a connection between the RMS and the constant force and non-fatiguing muscular contractions. For feature extraction, the RMS technique is commonly employed, because it is fast and economical in terms of computing while maintaining critical data [14].

$$RMS = \sqrt{\frac{1}{N}\sum_{i=1}^{N} |x_i|^2} \quad (6)$$

(vii) Waveform Length (WL)

Waveform length is the term used to describe the length of the waveform overall (WL). The amplitude, frequency, and duration of the waveform are calculated using the WL method [14].

$$WL = \sum_{i=1}^{N} |x_{i+1} - x_i| \quad (7)$$

Frequency domain

To extract features in the frequency domains, a signal's power spectral density is employed (PSD).

(i) Autoregressive Coefficients (AR)

It consists of the previous samples added to an error term for white noise [15].

$$x_n = \sum_{i=1}^{p} a_i x_{n-i} + w_n \quad (8)$$

where x_n represents a sample of the model signal, a_i the AR coefficients, w_n is the white noise error term, and p is the order of the AR model.

(ii) Frequency Median (FMD)

Frequency Median (FMD) is a term used to describe a frequency distribution based on the power spectral density (PSD). To determine the frequency domain feature for EMG, there are two basic forms of PSD estimation: parametric and nonparametric. Parametric techniques treat the signal as the product of a linear system. The model of a system is not assumed in nonparametric techniques.

$$FMD = \frac{1}{2}\sum_{i=1}^{M} PSD \quad (9)$$

Effects on paraspinal muscles during low back pain

Lower back discomfort is caused by injury to the back muscles or tendons. It turned out to be extremely widespread. These effects will be noticed between L3 and L5 in the lumbar region of the spine. It has been demonstrated that electromyography signals are more effective than other techniques in differentiating between those with persistent low back pain and healthy individuals [16, 17]. The findings of Anthony R. Humphrey *et al*. demonstrated that EMG variables may be used to identify between patients with chronic back pain and healthy controls since the variable values differed considerably between the two groups [16].

A patient with an EMG variable half-width and an initial median frequency (IMF) above 49 Hz had back pain 5.8 times more often than others (p=0.014) and three times more often than those with a half-width greater than 56 Hz and no history of persistent LBP [18]. The activation of paraspinal muscles in persons with acute non-specific lower back pain was investigated using measurements of average EMG (AEMG), visual analogue scale (VAS), and finger-to-floor (DFTF) [19]. They discovered that paraspinal muscle activity changes after massage therapy due to variations in flexion and extension values. The EMG signal varied throughout prolonged periods of silent sitting; patients with cLBP showed less temporal variability (p=0.03), higher RPE (rating of perceived exertion), and equal spatial variation in muscle activity compared to healthy individuals. This shows cLBP patients have trouble in tolerating low levels of static muscular tension [20].

Within another study the patients' lumbar paraspinal muscle tone and stiffness were assessed using the Standard Error of Measurement (SEM), Smallest Real Difference (SRD), and Bland-Altman Analyses (BAA). The study proved that upper-level lumbar measurement was not as accurate as lower-level lumbar measurement [21]. Ultrasonic Shear-wave Elastography (USWE) can also reveal muscular stiffness. Muscle thickness and lumbar back muscle shear elastic modulus were utilised as dependent factors in a study, whereas height, age, body weight, and sex were used as independent variables [22]. During work, the soleus, vastus, and fibularis longus muscles of the lower extremities for both patients with and without a history of back pain experienced lumbar paraspinal fatiguing due to altering in the soleus muscle, where the postural reaction that keeps lower extremity function going occurs [23].

Shin-Yi Chiou *et al*. (2017) looked at the relationship between EMG frequency characteristics (derived from CWT-Continuous Wavelet Transform analysis) and patient self-reported disability ratings. The patients filled in the Roland-Morris Disability Questionnaire (RMDQ) in addition to collection of bilateral EMG activity from the erector spinae at levels L4 and T12. The back extensors underwent three brief MVICs (maximum voluntary isometric contractions) before the torque reading on the dynamometer was recorded. For 200 milliseconds, CWT was applied to the EMG signals of each muscle, focusing on the maximal torque generated during the MVICs. The findings demonstrated that peak power at T12 and L4 as well as peak power frequency were lower in patients than in healthy people. Similar correlations between RMDQ and the average energy ratio at T12 were discovered (r=0.63; p=0.012), indicating that the lower frequencies had a predominant energy distribution [24].

Zeng Ming Hao *et al*. performed the Sorensen test for soldiers with and without chronic low back pain (CLBP) and published a comparative study about muscle fatigue and asymmetry on lumbar muscles using paired and independent samples t-tests and spatial distribution of those by repetitive ANOVA (Analysis on Variance) during sustained contraction using HDEMG. From these data they concluded that CLBP patients featured less significant effects on muscle fatigue and asymmetry in both sides of erector spinae and also uneven spatial distribution when compared to healthy subjects [25].

Influence of paraspinal muscles during FRP

The "flexion-relaxation (F-R) phenomenon" occurs when electrical silence begins immediately in back muscles when the trunk is flexed to a certain degree. The spinal extensors relax completely in full flexion, allowing the spinal ligaments to supply the flexion torque. This happens because the tension in the spinal ligaments contributes significantly to the anterior shear stress on the lumbar vertebrae and increases the strain on the facet

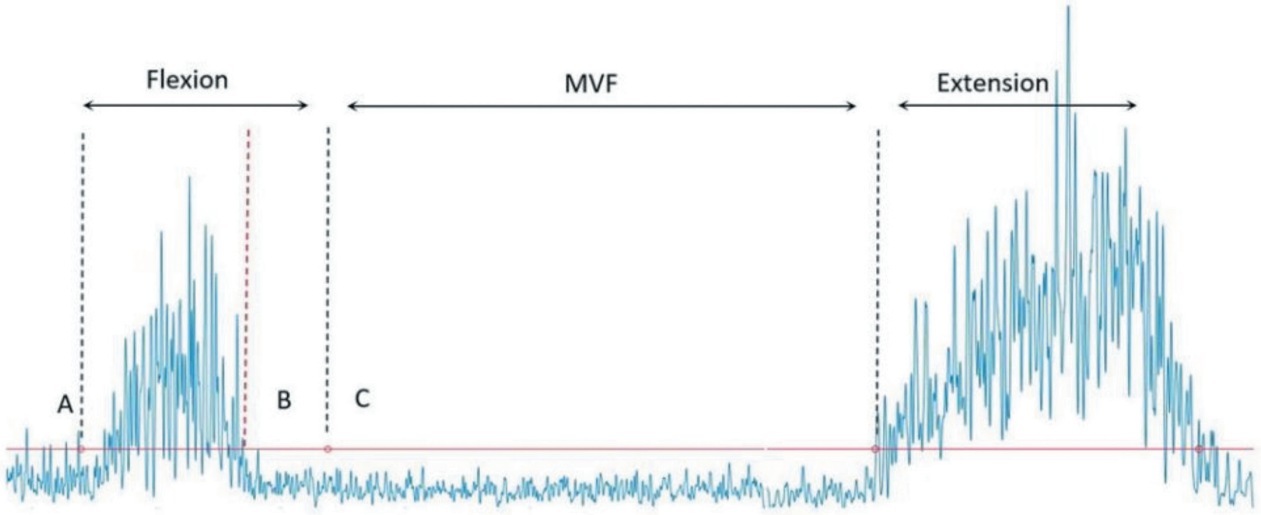


Fig 4. FRP of HDEMG

joints during weight training. Good technique is especially important when the spine is fully flexed [26].

Under spline statistical analysis, the paraspinal muscles with and without LBP had lower EMG amplitude with FRP than without FRP [19]. The erector spinae [ES] (L2, L5) muscle exhaustion caused with the Sorenson procedure was convincingly demonstrated in [9], and examination of the power spectrum suggested that it might affect FRP. They reached this conclusion by undertaking research to learn about the influence of FRP on earlier lumbar operations and their reaction to rehabilitation treatment [27].

P. Ippersiel *et al.* examined regional lumbopelvic coordination and flexion-relaxation patterns to better understand how individuals with and without low back pain perform during bending exercises. Regardless of movement duration, the hip/lower lumbar joint pair commonly moved out of phase in the low back pain group compared to the healthy group (mean difference = 24.7; 95 percent confidence interval = 3.93-45.4). The lower/upper lumbar coordination of the groups differed just a little. All muscles in the low back pain group exhibited decreased flexion-relaxation (average: 21.7%), with the multifidus showing the least relaxation after full flexion [28].

Anais Gouteron *et al.* carried out a detailed review on FRP (Figure 4) in the population with non-specific chronic low back pain (NSCLBP) in terms of prevalence, mean flexion-relaxation ratio (mFRR), mean extension-relaxation ratio (mERR), and distinction between asymptomatic and NSCLBP FRR. Their review concludes with the statement that the test was reproducible, sEMG can accurately identify an altered FRP in the NSCLBP group, and asymptomatic and NSCLBP FRP differed significantly in terms of FRR [29].

Impact of paraspinal muscles during several activities

EMG collected with surface electrodes was used to investigate the influence of paraspinal muscles owing to various activities. Experiments were conducted on paraspinal muscles to assess their atrophy, changes in CSA, and other effects of various activities such as physical exercise, massage treatment, static and dynamic training, and so on. L.A. Danneels *et al*. looked at the impact of paraspinal or paravertebral muscles during three distinct training modalities: stability, stabilization with dynamic resistance, and stabilization with static-dynamic resistance. According to the study, stabilizing exercise had no effect on muscle CSA, but rigorous lumbar resistance training was required to restore muscle size in cLBP patients with back muscles atrophy [30]. Table 2 enumerates experiments on various activities monitored to study paraspinal muscles changes.

Impact of paraspinal muscles found in imaging studies

LBP and other activities generate changes in the paraspinal muscles, which are investigated using imaging methods such as, CT, MRI and fMRI. Research exposed in Table 3 suggests that CSA and fatty infiltration in the paraspinal muscles are associated with disc herniation and spinal disorders. EMG data from people with acute and chronic lower back pain is being utilized to figure out what factors affect muscular fatigue. 3T MRI was employed in [46] to evaluate the geometric changes in CSA and contractile density following iatrogenic injury surgery. Based on changes in the atrophy of the back muscles, “stand-alone” oblique lateral interbody fusion (OLIF) and typical OLIF were compared in [47]. FCSA and Fatty Infiltration Percentage (FIP) were

Table 2. Experiments on various activities monitored to study paraspinal muscle changes.

Reference	Activity Monitored	Method	Result
[31]	Comparison of EMG features from Erector Spinae (ES) and Vastus Lateralis (VL) during bending forward and crouching down activities.	Median frequency (MNF) changes in the SEMG frequency domain index	Forward Bending: ES has higher significance than Right Vastus Lateralis Squatting down: Right Vastus Lateralis has higher Significance than ES
[32]	Monitoring myoelectric activity of paraspinal muscles for Idiopathic Scoliosis (IS) adolescents' habitual standing and sitting.	PUMC Type 1a and Type IIc subjects' root mean square (RMS) of SEMG reflects their spinal curvature state	A more consistent RMS SEMG ratio was seen in the trapezius, longissimus dorsi, and erector spinae after the physiotherapist suggested posture modification in the thoracic and lumbar areas. Thus, AIS patients were advised to use motor learning by practising recommended positions that help in balanced posture.
[33]	Alterations in the pattern of muscular activation affect spinal tissue creep when muscles are fatigued.	Surface electromyography was used to record the right and left erector spinae's large-array activity (EMG). The EMG root mean square (RMS), median frequency, and dispersion were compared on the x- and y-axes before and after the fatigue exercise.	The EMG median frequency was shown to alter significantly with muscular exhaustion.
[34]	The effect of repeated abrupt external perturbations on the trunk's neuromuscular adaptation after muscle fatigue.	A high-density EMG The activation of the erector spinal muscles was assessed using this method. A 3D motion analysis system was used to collect trunk kinematics from perturbation studies. Two-way measures were employed for baseline activity, reflex latency, reflex peak, and trunk kinematics to investigate adaptation, fatigue, and interaction effects.	Muscle activity was distributed more spatially in post-fatigue trials, compared to fatigue tasks. Values of baseline activity were higher in muscle fatigue and reduced in perturbation training.
[35]	Surface electromyography (SEMG) of the lumbar paraspinal muscles in stroke sufferers with later pulsion in a passive posture.	Correlation of Burke Lateropulsation scale during onset and duration of SEMG response.	In lateropulsion patients, paraspinal muscle activity had SEMG response length shorter on the weaker side after passive tilting at the stronger side, which implies more muscle activity during fast, passive tilting at the weaker side than controls. There is no correlation for dependent variables in BLS
[36]	Affect in lumbar reflex adaptation due to back muscle fatigue in response to fast external perturbations	Back muscular fatigue protocol (intermittent)	Erector Spinae (ES) reflex latencies were shorter by 25% (p<0.05) during expected versus unexpected conditions, To compensate for muscle weariness, a substantial external force disturbance would result in greater amplitude responses in paraspinal and even earlier activation.
[37]	The surrounding muscles of the cervical spine, thoracic spine, and lumbar spine are all affected by different designs of high heels.	The paraspinal muscles surrounding the C6, T7, and L5 vertebrae were an electrode.	Standing on wedge heels, setback heels, and French heels increased paraspinal activity in the cervical and lumbar spine as compared to bare feet. The various styles of heels, on the other hand, had no noticeable difference.

[38]	Dynamic superman contractions on stable and unstable surfaces, as well as unloaded body weight squats, activate the paraspinal muscles (longissimus and iliocostalis).	Correlation Analysis	Compared to Superman's exercise, the bodyweight squat produces equilibrium muscle activation in longissimus and iliocostalis on stable and unstable surfaces. Due to dynamic natures and daily activities, sports situation bodyweight squat was suggested, because it was better in activating paraspinal muscles than superman boat exercise.
[39]	Juchumseogi and Juchumseo Jireugi's motions affect paraspinal muscle activation.	Muscle activation was measured from C3, T7, and L3 during these motions. The movements were repeated three times, with the average results utilized in the analysis.	In comparison to simple standing, Juchumseogi and Juchumseo Jireugi movements increase paraspinal muscle activity in C3 and T7. When compared to Juchumseogi alone, Juchumseo Jireugi's motions In C3, T7, and L3, there was a considerable increase in muscular activity of paraspinal muscles. In C3, T7, and L3, the paraspinal muscular activation caused by Juchumseo Jireugi's motion was much greater than that caused by standing and Juchumseogi alone.
[40]	The effect of the bridge workout approach on rectus abdominis muscle change and paraspinal muscle activation while walking on the treadmill in high heels.	In the supine position, bridge exercise was done in a hook lying posture, whereas in the prone position, bridge exercise was done in a plank position. The rectus abdominis muscle's strength was measured by keeping the same posture for a certain amount of time. EMG (4D-MT & EMD-11, Relive, Korea) was utilized to evaluate paraspinal muscle activation.	The rectus abdominis muscle strength rises in the supine and prone groups during the bridge exercise. During bridge training, both groups' paraspinal muscular activation in the thoracic and lumbar portions diminishes.
[41]	During back bridge training, EMG was measured in the time and frequency domains from the Lumbar Multifidus (MF) and Erector Spinae (ES).	Between the first and last epochs of the test, the normalized Root Mean Square (RMS) value and the spectral Median Frequency (MF) were compared. Throughout the test, the dynamics of MF were acquired using the Short-Time Fourier Transform (STFT)	The LM muscle exhibited a bigger MF than the ES muscle towards the conclusion of the exercise, which remained constant. The slope of MF, on the other hand, was crucial for LM.
2009 [42]	The lumbar muscles react by creating tension-relaxation of viscoelastic tissue when undergoing extended passive cyclic trunk flexion and extension.	Passive exercise session	Decrease in posterior viscoelastic tissue over time due to supply of tension when muscle activity remains constant. After a passive flexion session, active flexion results in a rise in paraspinal muscle EMG and an increase in median frequency.
[43]	Lumbar Muscle Activation During Resistant and Non-Resistant Core Strength Exercises: EMG Analysis (Trunk extension exercise, superman boat exercise, Quadruped exercise)	To normalize EMG, the Root Mean Square (RMS) and Maximum Voluntary Contraction (MVC) methods were applied (per cent MVC)	Muscle activation during manually resisted and unresisted tasks is identical, according to the findings. During resistive training, the muscles were not certainly overused or strained. As a result, specialized muscle group resistance training can be used to develop target muscles.

[44]	Myofascial release	(1) superficial myofascial release; (2) deep myofascial release; 10 sessions, each lasting 40 minutes were employed in a ten-session, twice-weekly myofascial release technique intervention regimen. The Toe-Touch Test and electromyography activity were used to gauge the lumbar erector spinae muscle's flexibility and activity during trunk extension-flexion motion (EMG).	EMG activity in the right iliocostalis (p = 0.179; r = 0.43), right longissimus (p = 0.877; r = 0.05), left iliocostalis (p = 0.386; r = 0.29), and left longissimus (p = 0.418; r = 0.27) muscles was unaffected by myofascial release methods.
[45]	After lumbar spine surgery, patients may experience spinal muscular atrophy (PMA).	The search for research that documented PMA after spine surgery was conducted in the literature. The amount of PMA released following surgery was compared in three groups: lumbar fusion vs. nonfusion procedures, posterior vs. anterior lumbar fusion, and minimally invasive (MIS) posterior lumbar decompression and/or fusion vs. non-MIS equivalent therapy.	The mean postoperative volumetric PMA following fusion procedures was significantly higher than the mean postoperative volumetric PMA following non-fusion procedures (p=.0001), the mean postoperative volumetric PMA following posterior fusion procedures was significantly higher than the mean postoperative volumetric PMA following anterior fusion procedures (p=.0001), and the mean postoperative volumetric PMA following conventional fusions was significantly higher than the mean postoperative volumetric PMA following MIS fusions (p=.001). The mean volumetric lumbar PMA did not differ statistically between MIS and non-MIS decompression (p=.56). Postoperative PMA was shown to be higher following non-MIS fusions, posterior surgeries, and lumbar spine fusions.

measured using computed tomography (CT) before and after multifidus and erector spinae surgery. The automated threshold technique was also employed in research of paraspinal muscles morphology and fatty infiltration, with the results comparing the automatic approach to a novel thresholding method leading to the conclusion that the automatic method is more reliable [7].

Discussion

EMG characteristics in LBP

A subset of persons who are more prone to experience future low back pain can be identified using the lumbar paraspinal muscles' EMG features. We can notice that to varying degrees, all the EMG variables were able to discriminate between low back pain patients and healthy controls. The previous history of patients could not be distinguished from that of healthy controls or patients with persistent low back pain due to the small sample size of the history group or the wide range of the prior history group's variables. Back pain risk rose 5.8-fold when IMF was higher than 49 Hz (p = 0.014) [18]. Reduced peak power (T12 and L4) and decreased peak frequency may be seen in the patient's EMG signal characteristics at the L4-T12 levels (T12) [24]. It was shown that those with acute non-specific lower back

Table 3. CSA and fatty infiltration in the paraspinal muscles are associated with disc herniation and spinal disorders.

Reference	Objective	Materials And Methods	Inference
[48]	The link between spinal stenosis, lumbar paraspinal muscle atrophy, and lumbar disc herniation	Materials: Pattern of disc herniation and degree of spinal stenosis. Method: Investigation of Muscle atrophy grade (Multifidus, Longissimus, and Iliocostalis) and laterality.	79% of individuals with disc herniation had multifidus atrophy and they had no relationship between paraspinal muscle atrophy with disc herniation (p=0.15) and with any grade of spinal stenosis (p<0.01). Paraspinal muscle atrophy was found in 90% of individuals with spinal stenosis.

[49]	Functional magnetic resonance imaging [blood oxygen level-dependent (BOLD) imaging and T2 mapping] was used to examine the excitation of the lumbar paraspinal muscles before and after exercise.	Simple Roman Chair made of wood. CSA, R2*, and T2 values were measured in the iliocostalis, longissimus, and multifidus anatomical portions of the lower back during an upper-body flexion and extension exercise in that chair. The data was analyzed using SPSS 2.0 statistical software.	R2* was reduced (p<0.01), although CSA and T2 were higher after exercise. Although there was no statistical difference, males and females had substantially different R2*, CSA, and T2 values in the iliocostalis (p<0.05). In comparison to the multifidus and longissimus, the iliocostalis exhibited a higher overall CSA (p<0.05).
[50]	The goal of this study was to determine the skeletal muscle mass index (SMI) and paraspinal muscle composition in patients who had spinal surgery for lumbar spinal stenosis (LSS) or adult spinal deformity (ASD), as well as to evaluate if paraspinal muscles influence low back pain in ASD patients. (3) Determine which ASD spinal metrics are substantially impacted by paraspinal muscles on radiographs.	Dual Energy Absorptiometry was used to perform T2-weighted MRIs at the L3/L4 level. Methods: Paraspinal muscle rm CSAs were measured using preoperative T2w MRIs at the L3/4 level. Using dual-energy x-ray absorptiometry, the body's total bone mineral density and lean, soft tissue mass were calculated. The quantity of lean soft tissue in the appendix (kg/m^2) was used to compute the SMI (upper and lower limbs). For 110 consecutive patients with ASD and 50 consecutive patients with LSS who had spinal surgery, the Roland-Morris Disability Questionnaire, Oswestry Disability Index, spinopelvic characteristics, and rmCSA were assessed.	The overall SMI and sarcopenia morbidity rates were not different between LSS and ASD patients. The multifidus/erector muscles' CSA and rmCSA are negatively correlated with the Oswestry Disability Index, but the multifidus/erector muscles' CSA and sacral slope are positively correlated.
[51]	Using digital data from lumbar spine MRIs of patients with both acute and chronic low back pain, researchers looked at changes in the cross-sectional area (CSA) and composition of the paraspinal muscles (LBP)	The amount of fat infiltration was assessed using CSAs and the mean signal intensities of the bilateral paraspinal muscles (psoas major, PM, quadratus lumborum, QL, multifidus, MF, and erector spinae, ES). Results were contrasted between painful and non-painful groups as well as between different groups. The CSA and mean signal intensity of the MF muscle were evaluated in 42 patients with chronic unilateral LBP at three distinct levels: problem, above issue, and below issue.	In terms of pain, the chronic LBP group had considerably lower CSAs of MF and ES muscles compared to the acute LBP group, which had significantly lower CSAs of PM and ES muscles. In the chronic LBP group, measurements were made of the mean signal intensity and the amount of fat in the ES muscle on the afflicted side.
[52]	A complete muscle measuring procedure the worth able quality and agreement of related paraspinal muscle composition metrics were also discussed and acquired using OsiriX and ImageJ, two widely used image processing tools.	Materials: Researchers used axial T2-weighted magnetic resonance scans of the multifidus muscle, the erector spinae muscle, and the two muscles united at L4–L5, as well as lumbar magnetic resonance imaging on 30 patients at L4–L5. Method: Measurement of muscle CSA and composition. Each software package was used twice, at least 5 days apart, to duplicate all measurements. All prior measurements were hidden from the subject.	OsiriX or ImageJ was used to determine interrater reliability and standard error of measurement (SEM), with coefficients of reliability (intraclass correlation coefficients [ICCs]) ranging from .77 to .99 for OsiriX and .78 to .99 for ImageJ, respectively. The two software tools had comparable high levels of agreement when measuring muscle composition (inter-software ICCs of .81– .99).

[53]	Evaluating the postoperative lumbar spine	Materials: X-rays, flexion-extension x-rays, CT scans, myelography, and MRI scans were used. Methods: Most patients get one or more imaging tests, depending on the kind of first surgery and symptoms.	For adequate evaluation and selection of the radiological approach to be employed, knowledge of different types of surgical operations, instruments, typical postoperative changes, and probable problems was required.
[54]	Intra- and inter-measurement errors in paraspinal muscle parameters of functional cross-sectional area (FCSA), density, and T2 signal intensity were assessed using computed tomography (CT) and magnetic resonance imaging (MRI) (MRI).	Materials: In 29 patients with incessant low back pain, CT scan density and MRI T2 signal intensity of paraspinal muscles at L3-L4, L4-L5, and L5-S1 were compared. Methods: To assess intra- and interobserver reliability, two professionals. In three weeks, musculoskeletal radiologists and one superior spine surgeon tracked the area of interest twice.	FCSA has a reasonable to outstanding intraclass correlation value, while fatty infiltration has a good to the excellent intraclass correlation coefficient. Similarly, the inter-reliability ICCs are FCSA-fair to excellent and fatty infiltration-good to outstanding. Poor to excellent ICCs for CT and MRI scans The fatty infiltration measurement's relative standard error varied.
[55]	Quantitative and qualitative assessment of paraspinal muscles composition measurement (CSA) in association with maximum isokinetic lifting performance from routine lumbar spine MRI	Materials: MRI of paraspinal muscles at L3-L levels. Methods: The major outcome variables for isokinetic lifting power and effort, as well as body fat percentage, was relative and evaluative muscle constitutions analyses.	The CSAs and relative muscle constitution values based on cerebrospinal fluid adjusted signal intensity at the L3-L4 level were excellent (ICC = 0.95-0.99 and 0.96-0.99, respectively), while the evaluative muscle constitution evaluations (Kappa = 0.54-0.76) were only fair. According to the weak correlations between isokinetic lifting power and work and the relative and evaluative assessments of muscle constitution (r = 0.02-0.41), the evaluative estimations were preferred.

pain (ANLP) have different paraspinal muscle myoelectric activity signal amplitudes than healthy individuals. Consequently, the ANLP patients' myoelectric activity was recovered. In order to determine if patients with cLBP had a lower tolerance for low-level static muscular strain, the lumbar muscles alterations in the right and left side of patients were compared with healthy controls [20]. The study on Myo tonometer concluded that utilizing it in the clinical environment in young people with persistent LBP was useful. Upper lumbar level measurements were not as accurate as those performed at lower lumbar level [21]. Some studies concluded that HDEMG, Ultrasound Elastography (SWE) may help in understanding changes in stiffness and muscle mass [22, 23].

FRP impact on paraspinal muscles

The FRP was studied using HDEMG and Sorenson protocol, leading to the conclusion that people with LBP and FRP had a delayed onset. The presence of erector spinae muscle exhaustion also changes the FRP [26]. The changed FRP is frequently detected in the NSCLP population, and the test is quite repeatable. All muscles of the group of lumbar muscles showed decreased flexion-relaxation during complete flexion in the low back pain [9, 27] indicates FRP helps in knowing the conditions of the tissue due to excessive loading conditions (i.e. LBP).

Paraspinal muscles during several activities

LSS (Lumbar Spinal Stenosis) has previously been linked to impairments in lumbar movement perception. Recent studies validate the earlier conclusions using new patient data and controls that are matched for age. The disparity between the present LSS (Lumbar Spinal Stenosis) outcomes and the past disruption of local nervous and muscular tissues that resembled CLBP but was frequently more vigorous in LSS than CLPB may be explained by a variety of factors. Furthermore, stenotic patients reported leg and back pain, but CLBP patients often just experienced back pain. It was necessary to consider things like sensory loss that resulted in erroneous input, an information processing impairment, or a combination of the two to understand the variables that led to impaired lumbar movement perception. In order to achieve the proper

lumbosacral spine alignment, muscle spindles appear to be essential, because lumbar discomfort reduces muscle spindle input [56, 57]. As lumbar paraspinal muscle denervation and lumbar degeneration develop, proprioceptive information is likely to be lost [58]. LBP sufferers have reportedly trouble with their ability to fine-tune their postural balance regulation [59]. Reduced lumbar proprioception was previously demonstrated to be a reversible feature in sciatica patients who were followed for three months [60]. As a result (Figure 5), looking at the paraspinal muscles during various activities can aid to assess CSA, muscular atrophy, and fatty infiltration.

Paraspinal muscles examination by imaging

The MRI data aid in determining the pattern of disc herniation and the degree of spinal stenosis, both of which contribute to the development of lumbar paraspinal muscular atrophy. 90 percent of individuals with spinal stenosis had paraspinal muscle atrophy [31, 37]. To ascertain the activation of the lumbar paraspinal muscles before and after training, the SPSS 2.0 statistical tool was used in the screening of movement activities, such as upper body flexion and extension in a simple roman chair. Values for the cross-sectional area (CSA), R2* (rate of darkening), and T2 (rate of tranverse relaxation) of were calculated [32]. It was discovered that the ODI (Oswestry Disability Index) significantly correlated negatively with the multifidus and erector muscles rmCSA [33]. Bilateral paraspinal muscles' fat infiltration ratio, CSA, and mean signal intensity were all assessed to compare the painful and non-painful sides [34]. Paraspinal muscles features may be quantified using the software packages Osirix and Image J [35]. Imaging can also aid with postoperative lumbar spine assessment [38]. The fatty degeneration extent in [61] aided in determining the mean densities of all paraspinal muscles for all age groups, which led to a better knowledge of fatty infiltration. Researchers in [62] used kinematic MRI analysis to examine the relationship between lumbar muscle deterioration and spinal degenerative diseases utilizing the

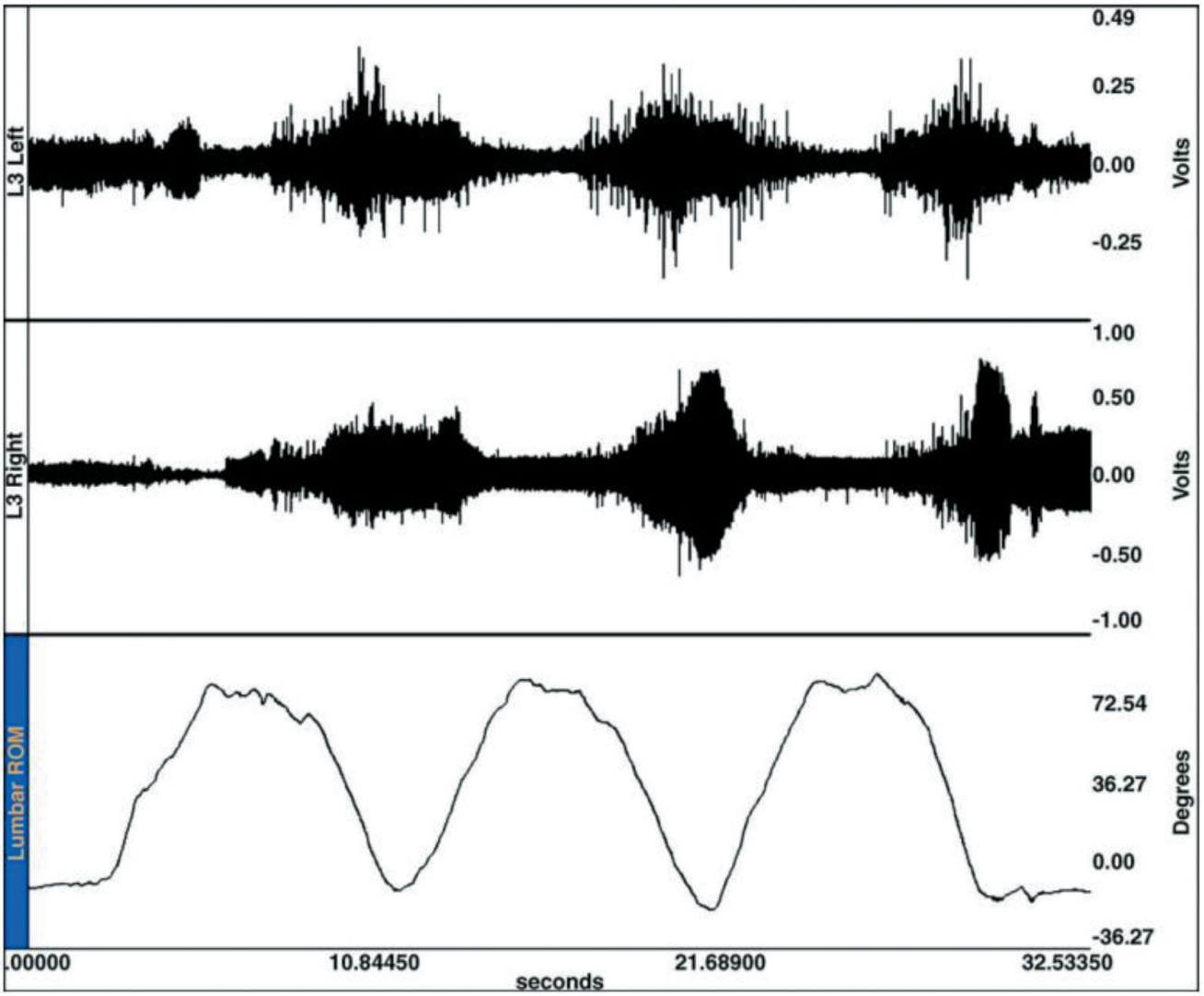


Figure 5. Flexion-extension phenomenon (C. J. COLLOCA & al., 2005 [27]).

Lumbar Indentation Value (LIV) as a quantitative variable and the Gout Allier classification as a qualitative variable. The authors of [63] conducted research on paraspinal muscle atrophy with or without internal screw fixation, using multivariate analysis to link age and fixation group. Fixation surgery was connected to higher muscle atrophy than non-fixation surgery [64]. Figure 6 shows the mean density of MF, ES, PS.

Conclusion

sEMG helps in acquiring a more precise knowledge of muscle activity variances. According to the data, CLBP atrophy was identified in the multifidus and paraspinal muscles, but not in the erector spinae. There was no evidence of atrophy in RLBP or ALBP. Fat infiltration was not observed in RLBP, however the results in CLBP were equivocal. In the paraspinal muscles, CLBP indicated no changes in fiber type. In individuals with any type of degenerative disc herniation, paraspinal muscular atrophy, which has a strong link to spinal stenosis, is prevalent. Because people with LBP seldom display signs of nerve or muscle damage, SEMG might provide essential information on evolution of the disease. Whether these metrics are practical and easy to gather in clinical settings, as well as which measurement combination is best for detecting LBP, requires more study. To better understand the causes of SEMG abnormalities in LBP and how to treat them, as well as to understand the significance of the association between the side of the disc herniation and unilateral or dominant paraspinal muscle atrophy on the same side, also requires further inquiry. This will enable these patients' therapeutic therapy to concentrate on workouts for unilateral muscle strengthening. Regional variations in the lumbar spine and the potential for subgroups with varied movement styles should both be considered when analyzing coordination in people with low back pain. Multifidus had the most flexion-relaxation changes; hence it should be considered while evaluating this construct.

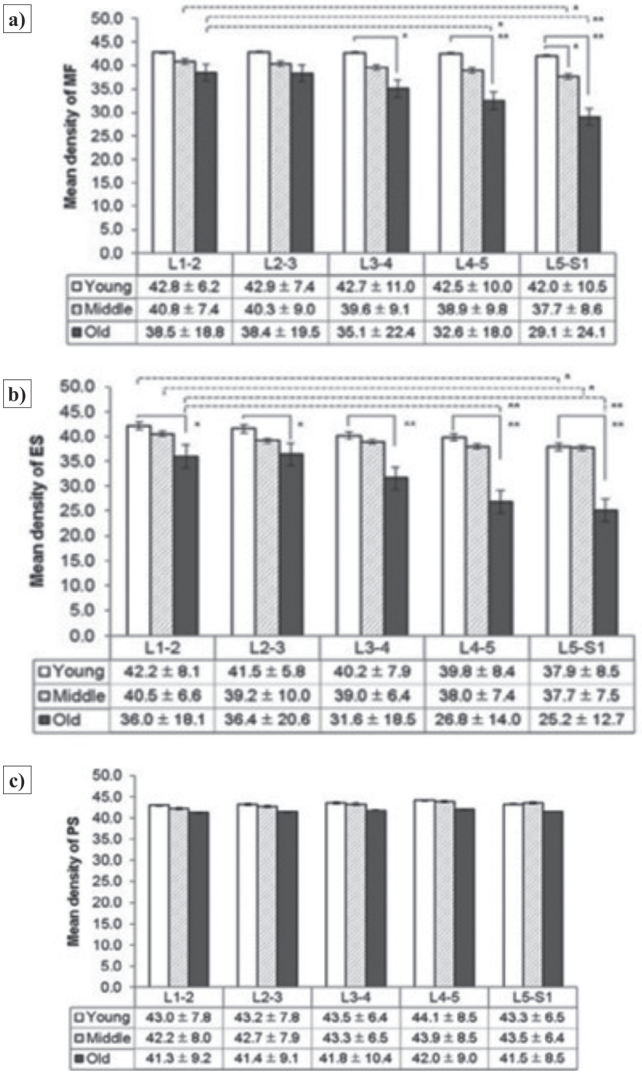


Figure 6. Mean density of (a) multifidus (MF) (b) erector spinae (ES) (c) psoas major (PS) (S.-H. LEE & al., 2015 [61])

Conflict of Interest

The authors have no conflict of interest to declare.

Acknowledgements

Not Applicable

Abbreviations

ANOVA	Analysis of Variance
AR	Autoregressive Coefficients
ASD	Adult Spinal Deformity
BAA	Bland-Altmann Analysis
BOLD	Blood Oxygen Level Dependent
cLBP	Chronic Low Back Pain
CNS	Central Nervous System
CSA	Cross Section Area
CT	Computer Tomography
CWT	Continuous Wavelet Transform
DECT	Dual Energy Computer Tomography
DFTF	Finger to Floor
EMG	Electromyography
ES	Erector Spinae
FCSA	Functional Cross-Sectional Area
FIP	Fatty Infiltration Package
FMD	Frequency Median
f-MRI	Functional Magnetic Resonance Imaging
FRP	Flexion-Relaxation Phenomenon
FRR	Flexion-Relaxation Ratio
HD-sEMG	High Density surface Electromyography
ICC	Intra Class Correlation
IEMG	Integrated Electromyography
IMF	Initial Median Frequency
IS	Idiopathic Scoliosis
L3-L4	3rd and 4th level of lumbar region in spine
LBP	Low Back Pain
LSS	Lumbar Spinal Stenosis
MAV	Mean Absolute Value
ODI	Oswestry Disability Index
MAVS	Mean Absolute Value Slope
mERR	Mean Extension-Relaxation Ratio
MF	Multifidus
mFRR	Mean Flexion-Relaxation Ratio

MIS	Minimally Invasive Surgery
MNF	Median Frequency
MRI	Magnetic Resonance Imaging
MU	Motor Units
MVF	Maximum Voluntary Force
MVIC	Maximum Voluntary Isometric Contraction
NSCLBP	Non-Specific Chronic Low Back Pain
OLIF	Oblique Lateral Interbody Fusion
PMA	Peroneal Muscle Atrophy
PSD	Power Spectral Density
PUMC	Peking Union Medical College
RMDQ	Roland-Morris Disability Questionnaire
RMS	Root Mean Square
SEM	Standard Error of Measurement
SMI	Skeletal Muscle Mass Index
SPSS	Statistical Package of Social Science
SRD	Smallest Real Difference
SSI	Simple Square Integral
STFT	Short Time Fourier Transform
USWE	Ultrasonic Shear-Wave Elastography
VAR	Variance
VAS	Visual Analogue Scale
VL	Vastus Lateralis
WL	Waveform length

References

1. Latif A, Zahara A, Cheema AS, et al. Backache: Presentation and Diagnosis: A Prospective Study at Allama Iqbal Memorial Teaching Hospital Sialkot. PJMH 2018 1; 12:89-93.
2. Sun D, Liu P, Cheng J, Ma Z, Liu J, Qin T. Correlation between intervertebral disc degeneration, paraspinal muscle atrophy, and lumbar facet joints degeneration in patients with lumbar disc herniation. BMC Musculoskelet Disord. 2017 Apr 20;18(1):167. doi: 10.1186/s12891-017-1522-4. PMID: 28427393; PMCID: PMC5399427.
3. Tony BJAR, Alphin MS, Sri Krishnan G. Analysis of upper body ergonomic parameters on commuter motorbike users, J. Transp. Heal, 2020;16:100828.
4. Berry DB, Padwal J, Johnson S, Parra CL, Ward SR, Shahidi B. Methodological considerations in region of interest definitions for paraspinal muscles in axial MRIs of the lumbar spine. BMC Musculoskelet Disord. 2018 May 7;19(1):135. doi: 10.1186/s12891-018-2059-x. PMID: 29734942; PMCID: PMC5938809.
5. Drost G, Stegeman DF, van Engelen BG, Zwarts MJ. Clinical applications of high-density surface EMG: a systematic review. J Electromyogr Kinesiol. 2006 Dec;16(6):586-602. doi: 10.1016/j.jelekin.2006.09.005. PMID: 17085302.
6. Farina D, Cescon C, Merletti R. Influence of anatomical, physical, and detection-system parameters on surface EMG. Biol Cybern. 2002 Jun;86(6):445-56. doi: 10.1007/s00422-002-0309-2. PMID: 12111273.
7. Fortin M, Omidyeganeh M, Battié MC, Ahmad O, Rivaz H. Evaluation of an automated thresholding algorithm for the quantification of paraspinal muscle composition from MRI images. Biomed Eng Online. 2017 May 22;16(1):61. doi: 10.1186/s12938-017-0350-y. PMID: 28532491; PMCID: PMC5441067.
8. Champagne A, Descarreaux M, Lafond D. Back and hip extensor muscles fatigue in healthy subjects: task-dependency effect of two variants of the Sorensen test. Eur Spine J. 2008 Dec;17(12):1721-6. doi: 10.1007/s00586-008-0782-y. Epub 2008 Sep 24. PMID: 18813961; PMCID: PMC2587667.
9. Neblett R, Mayer TG, Brede E, Gatchel RJ. The effect of prior lumbar surgeries on the flexion relaxation phenomenon and its responsiveness to rehabilitative treatment. Spine J. 2014 Jun 1;14(6):892-902. doi: 10.1016/j.spinee.2013.07.442. Epub 2013 Nov 15. PMID: 24246746.
10. Arjunan S, Kumar D, Poon W, Rudolph H, Hu Y. Variability in Surface Electromyogram During Gait Analysis of Low Back Pain Patients, J. Med. Biol. Eng. 2010;30:133–138.
11. Tony BJ, Alphin MS. Biomechanical Response of the Human Foot Model Exposed to Vibrations: A Finite Element Analysis. J. Biomater. Tissue Eng, 2021;11:2097–2108.
12. Alkan A, Günay M. Identification of EMG signals using discriminant analysis and SVM classifier. Expert Syst. Appl. 2012;39(1):44–47.
13. OSKOEI MA. HU H. GA-based Feature Subset Selection for Myoelectric Classification, in 2006 IEEE International Conference on Robotics and Biomimetics, 2006:1465–1470.
14. Veer K, Sharma T. Extraction and Analysis of above elbow SEMG for Pattern classification, J. Med. Eng. Technol. 2016;40(4):149–154.
15. Tony BJAR, Alphin MS, Velmurugan D. Influence of handle shape and size to reduce the hand-arm vibration discomfort. Work. 2019;63(3):415-426. doi: 10.3233/WOR-192948. PMID: 31256108.
16. Humphrey AR, Nargol AV, Jones AP, Ratcliffe AA, Greenough CG. The value of electromyography of the lumbar paraspinal muscles in discriminating between chronic-low-back-pain sufferers and normal subjects. Eur Spine J. 2005 Mar;14(2):175-84. doi: 10.1007/s00586-004-0792-3. Epub 2004 Nov 12. PMID: 15549487; PMCID: PMC3476699.
17. Oddsson LI, De Luca CJ. Activation imbalances in lumbar spine muscles in the presence of chronic low back pain. J Appl Physiol (1985). 2003 Apr;94(4):1410-20. doi: 10.1152/japplphysiol.01183.2001. Epub 2002 Dec 13. PMID: 12482760.

18. Heydari A, Nargol AV, Jones AP, Humphrey AR, Greenough CG. EMG analysis of lumbar paraspinal muscles as a predictor of the risk of low-back pain. Eur Spine J. 2010 Jul;19(7):1145-52. doi: 10.1007/s00586-010-1277-1. Epub 2010 Feb 2. PMID: 20127260; PMCID: PMC2900021.
19. Qiao J, Zhang SL, Zhang J, Feng D. A study on the paraspinal muscle surface electromyography in acute nonspecific lower back pain. Medicine (Baltimore). 2019 Aug;98(34):e16904. doi: 10.1097/MD.0000000000016904. PMID: 31441870; PMCID: PMC6716703.
20. Krishnan N, Alphin MS, Doss TTB, Biomechanical analysis and comparison of actual lumbarbone with lumbar bone prototype. Biomedical Research. 2017;28:2519–2523.
21. Hu X, Lei D, Li L, Leng Y, Yu Q, Wei X, Lo WLA. Quantifying paraspinal muscle tone and stiffness in young adults with chronic low back pain: a reliability study. Sci Rep. 2018 Sep 25;8(1):14343. doi: 10.1038/s41598-018-32418-x. PMID: 30254233; PMCID: PMC6156595.
22. Masaki M, Aoyama T, Murakami T, Yanase K, Ji X, Tateuchi H, Ichihashi N. Association of low back pain with muscle stiffness and muscle mass of the lumbar back muscles, and sagittal spinal alignment in young and middle-aged medical workers. Clin Biomech (Bristol, Avon). 2017 Nov;49:128-133. doi: 10.1016/j.clinbiomech.2017.09.008. Epub 2017 Sep 14. PMID: 28934633.
23. Bunn EA, Grindstaff TL, Hart JM, Hertel J, Ingersoll CD. Effects of paraspinal fatigue on lower extremity motoneuron excitability in individuals with a history of low back pain. J Electromyogr Kinesiol. 2011 Jun;21(3):466-70. doi: 10.1016/j.jelekin.2011.02.002. Epub 2011 Mar 8. PMID: 21388827.
24. Chiou SY, Koutsos E, Georgiou P, Strutton PH. Association between spectral characteristics of paraspinal muscles and functional disability in patients with low back pain: a cohort study. BMJ Open. 2018 Feb 14;8(2):e017091. doi: 10.1136/bmjopen-2017-017091. PMID: 29444776; PMCID: PMC5829836.
25. Hao Z, Xie L, Wang J, Hou Z. Spatial Distribution and Asymmetry of Surface Electromyography on Lumbar Muscles of Soldiers with Chronic Low Back Pain. Pain Res Manag. 2020 Oct 26;2020:6946294. doi: 10.1155/2020/6946294. PMID: 33163126; PMCID: PMC7605927.
26. Murillo C, Martinez-Valdes E, Heneghan NR, Liew B, Rushton A, Sanderson A, Falla D. High-Density Electromyography Provides New Insights into the Flexion Relaxation Phenomenon in Individuals with Low Back Pain. Sci. Rep. 2019;9(1):15938. doi: 10.1038/s41598-019-52434-9.
27. Colloca CJ, Hinrichs RN. The biomechanical and clinical significance of the lumbar erector spinae flexion-relaxation phenomenon: a review of literature. J Manipulative Physiol Ther. 2005 Oct;28(8):623-31. doi: 10.1016/j.jmpt.2005.08.005. PMID: 16226632.
28. Ippersiel P, Preuss R, Fillion A, Jean-Louis J, Woodrow R, Zhang Q, Robbins SM. Inter-joint coordination and the flexion-relaxation phenomenon among adults with low back pain during bending. Gait Posture. 2021 Mar;85:164-170. doi: 10.1016/j.gaitpost.2021.02.001. Epub 2021 Feb 5. PMID: 33581560.
29. Gouteron A, Tabard-Fougère A, Bourredjem A, Casillas JM, Armand S, Genevay S. The flexion relaxation phenomenon in nonspecific chronic low back pain: prevalence, reproducibility and flexion-extension ratios. A systematic review and meta-analysis. Eur Spine J. 2022 Jan;31(1):136-151. doi: 10.1007/s00586-021-06992-0. Epub 2021 Sep 22. PMID: 34553264.
30. Danneels LA, Vanderstraeten GG, Cambier DC, Witvrouw EE, Bourgois J, Dankaerts W, De Cuyper HJ. Effects of three different training modalities on the cross sectional area of the lumbar multifidus muscle in patients with chronic low back pain. Br J Sports Med. 2001 Jun;35(3):186-91. doi: 10.1136/bjsm.35.3.186. PMID: 11375879; PMCID: PMC1724339.
31. Xiao J, Gao J, Wang H, Liu K, and Yang X. The surface EMG characteristics between erector spinae and vastus lateralis during bending forward and squatting down tasks. Physiology Journal. 2015:537379.
32. Kwok G, Yip J, Cheung MC, Yick KL. Evaluation of Myoelectric Activity of Paraspinal Muscles in Adolescents with Idiopathic Scoliosis during Habitual Standing and Sitting. Biomed Res Int. 2015;2015:958450. doi: 10.1155/2015/958450. Epub 2015 Oct 25. PMID: 26583151; PMCID: PMC4637075.
33. Abboud J, Nougarou F, Descarreaux M. Muscle Activity Adaptations to Spinal Tissue Creep in the Presence of Muscle Fatigue. PLoS One. 2016 Feb 11;11(2):e0149076. doi: 10.1371/journal.pone.0149076. PMID: 26866911; PMCID: PMC4750977.
34. Abboud J, Nougarou F, Lardon A, Dugas C, Descarreaux M. Influence of Lumbar Muscle Fatigue on Trunk Adaptations during Sudden External Perturbations. Front Hum Neurosci. 2016 Nov 14;10:576. doi: 10.3389/fnhum.2016.00576. PMID: 27895569; PMCID: PMC5107576.

35. Babyar SR, McCloskey KH, Reding M. Surface electromyography of lumbar paraspinal muscles during seated passive tilting of patients with lateropulsion following stroke. Neurorehabil Neural Repair. 2007 Mar-Apr;21(2):127-36. doi: 10.1177/1545968306291857. PMID: 17312088.
36. Dupeyron A, Perrey S, Micallef JP, Pélissier J. Influence of back muscle fatigue on lumbar reflex adaptation during sudden external force perturbations. J Electromyogr Kinesiol. 2010 Jun;20(3):426-32. doi: 10.1016/j.jelekin.2009.05.004. PMID: 19595613.
37. Han D. Muscle activation of paraspinal muscles in different types of high heels during standing. J Phys Ther Sci. 2015 Jan;27(1):67-9. doi: 10.1589/jpts.27.67. Epub 2015 Jan 9. PMID: 25642040; PMCID: PMC4305600.
38. Carvalheiro Reiser F, Gonçalves Durante B, Cordeiro de Souza W, Paulo Gomes Mascarenhas L, Márcio Gatinho Bonuzzi G. Paraspinal muscle activity during unstable superman and bodyweight squat exercises. J Funct Morphol Kinesiol. 2017;2(1):9. doi: 10.3390/jfmk2010009.
39. Baek J, Lee J, Kim J, Kim J, Han D, Byun S. The effects of Juchumseogi and Juchumseo Jireugi motions of taekwondo on muscle activation of paraspinal muscles. J Phys Ther Sci. 2015 Sep;27(9):2793-6. doi: 10.1589/jpts.27.2793. Epub 2015 Sep 30. PMID: 26504295; PMCID: PMC4616096.
40. Kang T, Lee J, Seo J, Han D. The effect of bridge exercise method on the strength of rectus abdominis muscle and the muscle activity of paraspinal muscles while doing treadmill walking with high heels. J Phys Ther Sci. 2017 Apr;29(4):707-712. doi: 10.1589/jpts.29.707. Epub 2017 Apr 20. PMID: 28533614; PMCID: PMC5430277.
41. Mello RG, Carriço IR, da Matta TT, Nadal J, Oliveira LF. Lumbar multifidus and erector spinae electromyograms during back bridge exercise in time and frequency domains. J Back Musculoskelet Rehabil. 2016;29(1):123-33. doi: 10.3233/BMR-150607. PMID: 26406188.
42. Olson MW, Li L, Solomonow M. Interaction of viscoelastic tissue compliance with lumbar muscles during passive cyclic flexion-extension. J Electromyogr Kinesiol. 2009 Feb;19(1):30-8. doi: 10.1016/j.jelekin.2007.06.011. Epub 2007 Aug 20. PMID: 17703955.
43. Sivasubramanian S, Srinivasan P, Baala N, Karunakaran S. Emg Analysis Of Lumbar Muscle Activations During Resisted And Unresisted Core Strength Exercises. Int. J. Biomed. Clin. Eng. 2020;9:12–24.
44. Rodrigues L, Freitas Sant'Anna PC, La Torre M, Dhein W. Effects of myofascial release on flexibility and electromyographic activity of the lumbar erector spinae muscles in healthy individuals. J Bodyw Mov Ther. 2021 Jul;27:322-327. doi: 10.1016/j.jbmt.2021.03.015. Epub 2021 Mar 25. PMID: 34391252.
45. Arash E. Minimally Invasive Transforaminal Lumbar Interbody Fusion in the Outpatient Setting. Orthopedics. 2016;39(6):e1218–e1222.
46. Ghiasi MS. Cross-sectional area of human trunk paraspinal muscles before and after posterior lumbar surgery using magnetic resonance imaging. Eur. Spine J. 2016;25(3):774–782.
47. He W, He D, Sun Y, Xing Y, Liu M, Wen J, Wang W, Xi Y, Tian W, Ye X. Quantitative analysis of paraspinal muscle atrophy after oblique lateral interbody fusion alone vs. combined with percutaneous pedicle screw fixation in patients with spondylolisthesis. BMC Musculoskelet Disord. 2020 Jan 14;21(1):30. doi: 10.1186/s12891-020-3051-9. PMID: 31937277; PMCID: PMC6961348.
48. He K, Head J, Mouchtouris N, Hines K, Shea P, Schmidt R, Hoelscher C, Stricsek G, Harrop J, Sharan A. The Implications of Paraspinal Muscle Atrophy in Low Back Pain, Thoracolumbar Pathology, and Clinical Outcomes After Spine Surgery: A Review of the Literature. Global Spine J. 2020 Aug;10(5):657-666. doi: 10.1177/2192568219879087. Epub 2019 Oct 9. PMID: 32677568; PMCID: PMC7359686.
49. Huang YL, Zhou JL, Jiang YM, Zhang ZG, Zhao W, Han D, He B. Assessment of lumbar paraspinal muscle activation using fMRI BOLD imaging and T2 mapping. Quant Imaging Med Surg. 2020 Jan;10(1):106-115. doi: 10.21037/qims.2019.10.20. PMID: 31956534; PMCID: PMC6960437.
50. Katsu M, Ohba T, Ebata S, Oba H, Koyama K, Haro H. Potential Role of Paraspinal Musculature in the Maintenance of Spinopelvic Alignment in Patients With Adult Spinal Deformities. Clin Spine Surg. 2020 Mar;33(2):E76-E80. doi: 10.1097/BSD.0000000000000862. PMID: 31385849.
51. Wan Q, Lin C, Li X, Zeng W, Ma C. MRI assessment of paraspinal muscles in patients with acute and chronic unilateral low back pain. Br J Radiol. 2015 Sep;88(1053):20140546. doi: 10.1259/bjr.20140546. Epub 2015 Jun 24. PMID: 26105517; PMCID: PMC4743557.
52. Fortin M, Battié MC. Quantitative paraspinal muscle measurements: inter-software reliability and agreement using OsiriX and ImageJ. Phys Ther. 2012

Jun;92(6):853-64. doi: 10.2522/ptj.20110380. Epub 2012 Mar 8. PMID: 22403091.

53. Herrera IH, Moreno de la Presa RM, Gutiérrez RG, Ruiz EB, García Benassi JMG. Evaluación de la columna lumbar posquirúrgica. Radiologia. 2013;55:12–23. doi: 10.1016/j.rx.2011.12.004.
54. Hu ZJ, He J, Zhao FD, Fang XQ, Zhou LN, Fan SW. An assessment of the intra- and inter-reliability of the lumbar paraspinal muscle parameters using CT scan and magnetic resonance imaging. Spine (Phila Pa 1976). 2011 Jun;36(13):E868-74. doi: 10.1097/BRS.0b013e3181ef6b51. PMID: 21224757.
55. Ropponen A, Videman T, Battié MC. The reliability of paraspinal muscles composition measurements using routine spine MRI and their association with back function. Man Ther. 2008 Aug;13(4):349-56. doi: 10.1016/j.math.2007.03.004. Epub 2007 Jun 6. PMID: 17556006.
56. Brumagne S, Cordo P, Lysens R, Verschueren S, Swinnen S. The role of paraspinal muscle spindles in lumbosacral position sense in individuals with and without low back pain. Spine (Phila Pa 1976). 2000 Apr 15;25(8):989-94. doi: 10.1097/00007632-200004150-00015. PMID: 10767813.
57. Mok NW, Brauer SG, Hodges PW. Postural recovery following voluntary arm movement is impaired in people with chronic low back pain. Gait Posture. 2011 May;34(1):97-102. doi: 10.1016/j.gaitpost.2011.03.021. Epub 2011 May 6. PMID: 21530268.
58. Leinonen V, Määttä S, Taimela S, Herno A, Kankaanpää M, Partanen J, Hänninen O, Airaksinen O. Paraspinal muscle denervation, paradoxically good lumbar endurance, and an abnormal flexion-extension cycle in lumbar spinal stenosis. Spine (Phila Pa 1976). 2003 Feb 15;28(4):324-31. doi: 10.1097/01.BRS.0000048495.81763.8C. PMID: 12590204.
59. Fortin M, Lazáry À, Varga PP, Battié MC. Association between paraspinal muscle morphology, clinical symptoms and functional status in patients with lumbar spinal stenosis. Eur Spine J. 2017 Oct;26(10):2543-2551. doi: 10.1007/s00586-017-5228-y. Epub 2017 Jul 26. PMID: 28748488.
60. Leinonen V, Kankaanpää M, Luukkonen M, Kansanen M, Hänninen O, Airaksinen O, Taimela S. Lumbar paraspinal muscle function, perception of lumbar position, and postural control in disc herniation-related back pain. Spine (Phila Pa 1976). 2003 Apr 15;28(8):842-8. PMID: 12698130.
61. Lee SH, Son ES, Seo EM, Suk KS, Kim KT. Factors determining cervical spine sagittal balance in asymptomatic adults: correlation with spinopelvic balance and thoracic inlet alignment. Spine J. 2015 Apr 1;15(4):705-12. doi: 10.1016/j.spinee.2013.06.059. Epub 2013 Sep 8. PMID: 24021619.
62. Zecca M, Micera S, Carrozza MC, Dario P. Control of multifunctional prosthetic hands by processing the electromyographic signal. Crit Rev Biomed Eng. 2002;30(4-6):459-85. doi: 10.1615/critrevbiomedeng.v30.i456.80. PMID: 12739757.
63. Palpan Flores A, García Feijoo P, Isla Guerrero A. Paraspinal muscle atrophy after posterior lumbar surgery with and without pedicle screw fixation with the classic technique. Neurocirugia (Astur : Engl Ed). 2019 Mar-Apr;30(2):69-76. English, Spanish. doi: 10.1016/j.neucir.2018.11.006. Epub 2018 Dec 20. PMID: 30579798.
64. Kawaguchi Y, Matsui H, Tsuji H. Back muscle injury after posterior lumbar spine surgery. Part 1: Histologic and histochemical analyses in rats. Spine (Phila Pa 1976). 1994 Nov 15;19(22):2590-7. doi: 10.1097/00007632-199411001-00017. PMID: 7855686.

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3835-3840
doi: 10.25083/rbl/28.1/3835.3840

Received for publication, July, 28, 2023
Accepted, August, 14, 2023

Original article

A current review for some methodological aspects on using *Crocus sativus* and *Whitania somnifera* sp. extracts in the treatment of schizophrenia

PETRU FABIAN LUNGU[1], ALIN CIOBICA[1,2,3]

[1] Department of Biology, Faculty of Biology, Alexandru Ioan Cuza University, B dul Carol I, no 11, 700506 Iasi, Romania
[2] Center of Biomedical Research, Romanian Academy, B dul Carol I, no 8, 700505 Iasi, Romania
[3] Center of Biomedical Research, Romanian Academy, Iasi Branch, B dul Carol I, no. 8, 700506 Iasi, Romania

Abstract

Abstract: Schizophrenia is a severe mental illnes that affects population all around the world which poses a problem for the abilities of the affected person as well as their family members. Aim of the study: This narrative review has as its objective the potential evaluation of treatment with extracts from *Crocus sativus* and *Whitania somnifera* on patients with schizophrenia. Results: The plant extracts *C. sativus* and *W. somnifera* have a very well developed extraction method and beneficial effects in the case of patients with schizophrenia. Conclusion: *Crocus sativus and Whitania somnifera* extracts could be used as an adjuvant treatment for positive and negative simptoms in schizophrenia.

Keywords

Crocus sativus, *Whitania somnifera*, Plant extract.

To cite this article: LUNGU PF. A current review for some methodological aspects on using *Crocus sativus* and *Whitania somnifera* sp. extracts in the treatment of schizophrenia. *Rom Biotechnol Lett.* 2023; 28(1): 3835-3840 DOI: 10.25083/rbl/28.1/3835.3840

*Corresponding author: Alin Ciobica. email: alin.ciobica@uaic.ro

Introduction

Schizophrenia is a severe chronic mental illness that affects up to 1% of the global population. It's a complex and multifaceted psychiatric condition that hinders social, occupational, and individual functioning and results in a deterioration in the patients' standard of life. This condition typically appears in late adolescence or early adulthood. Positive symptoms (e.g., hallucinations, delusions, disordered thinking, catatonic behavior), negative symptoms (e.g., social withdrawal, anhedonia, avolition, neglect of hygiene), and cognitive disturbances (e.g., in attention, executive functioning, and memory) are common in schizophrenia [1, 2].

The mechanism and etiology of schizophrenia are still poorly understood. Even so, it is generally recognized as a composite neurodevelopmental disorder influenced by genetic and environmental factors [3, 4]. It has been discovered, in particular, that monozygotic siblings of schizophrenics have a 50–80% probability of contracting the illness. Additionally, aberrant synaptic connections between various brain regions and inadequate brain maturation are also demonstrated [5]. It's interesting to note that more and more studies are arguing that oxidative stress has a role in the pathophysiology of schizophrenia [2, 6].

According to clinical research, first-generation or atypical conventional antipsychotics have some efficacy in reducing positive symptoms but are ineffective in reducing negative symptoms and cognitive deficits in schizophrenia patients. However, many drugs have significant adverse effects that limit their usefulness. Particularly, negative effects on the motor system (Parkinsonism) are linked to the use of conventional neuroleptics (such as chlorpromazine and haloperidol). On the other hand, the usage of atypical antipsychotics (such as clozapine, olanzapine, and risperidone) results in weight gain rather than Parkinsonism. Additionally, 30% of patients are resistant to the therapy mentioned above. Collectively, these findings indicate that new drugs that could reduce the negative symptoms and cognitive deficiencies that are typical of schizophrenia patients are urgently needed [2, 7, 8].

The use of plant extracts and their bioactive elements as potential anti-schizophrenia drugs has recently been recommended as one of the many alternative therapies for the treatment of schizophrenia. In the current investigation, we aim to critically evaluate any potential therapeutic benefit of *Crocus sativus* and *Withania somnifera* extracts and their constituent parts for treating schizophrenia.

Methods

We have conducted searches for Crocus sativus and Withania somnifera extracts and their impact in pacients with schizophrenia. For the most part, information was taken from scientific journals, published between 2005 and 2022 about the different strategies and methods used in makeing extracts with the plants mentioned before. The search keywords used were „withania somnifera biological constituents"; „withania somnifera compounds"; „withania somnifera schizophrenia"; „crocus sativus extract schizophrenia"; „crocus sativus chemical constituents"; „crocus sativus extract".

Results

Crocus sativus L. (Saffron)

Crocus sativus L. (CS) is a perennial herb that belongs to the Liliaceae line of the Iridaceae family and the genus Crocus. Several nations, including Azerbaijan, China, France, Greece, Egypt, India, Iran, Israel, Italy, Mexico, Morocco, Spain, and Turkey, grow this plant. The final output of this plant is the spice saffron. The dried, deep-red stigmas of the CS flower are saffron, in filament form. Each bloom has three stigmata, each of which weighs about 2 mg. To obtain 1 kilogram of spice, 150.000 flowers must be carefully chosen. Saffron has a distinctive hue, flavor, and aroma. There have been many uses for saffron throughout history, from antiquity to the present. It is frequently used as a perfume and as a spice to flavor and color food and beverage preparations. Saffron is still typically consumed by adding it to food or any hot or warm beverage [9, 10].

Chemistry of CS

We can mention the stamens, perianth, and stigma as the chemical components of the plant. They contain the following:

- A total of 46 components were found in stamen of which 8 compounds were in a higher percentage 4-hydroxydihydro-2-(3H)-furanone (22.01%), hexadecanoic acid (12.09%), tyrosol (7.52%), benzeneacetic acid (5.23%), linolenic acid (4.96%), linoleic acid (3.86%), 1-docosene (3.85%), and vitamin E (3.63%) [11].
- For perianth a total of 50 compounds were found, from which 4 compounds were in a hihger percentage 4-hydroxydihydro-2(3H)-furanone (22.12%), hexadecanoic acid (18.14%), linolenic acid (7.73%) and stigmasterol (4.20%) [11].
- And for stigma 34 components of which 6 compunds were in higher percentage 1,3,3-trimethyl-2-vinyl-1-cyclohexene (22.36%), diisooctyl phthalate (14.77%),

hexadecanoic acid (9.48%), cis-9,cis-12-octadecadienoic acid (7.49%), 4-hydroxy3,5,5-trimethyl-2-cyclohexen-1 -one (4.74%) and stigmasterol (3.31%) [11].

The effect of CS extracts and different compunds in schizophrenia and depression

Norbala et al. created the pills from Novin Zaferan Co. in Mashhad, Iran, which donated the saffron utilized in this study, and the Department of Cultivation and Development at the Institute of Medicinal Plants in Tehran, Iran, recognized it. Stigma is the component of Crocus sativus that is used as an addition and a herbal remedy. The extract from the stigma was made as follows: In three steps of percolation, 120 g of dried and ground stigmas were combined with 1800 ml of 80% ethanol. The ethanolic extract was then dried by evaporation at a temperature between 35 and 40 °C. Each capsule contained lactose as a filler, magnesium stearate as a lubricant, dried saffron extract (15 mg), and sodium starch glycolate as a disintegrant [12, 13].

In this modest preliminary double-blind and randomized study, Norbala and his team compared the efficacy of saffron at a similar dosage to that of fluoxetine in the treatment of mild to moderate depression. The results showed no statistically important difference between the two substances [13].

The Fadai et al. clinical research, in which aqueous extract (SAE) and crocin were made using the procedure outlined in their prior investigations [14, 15, 16], it's the only one to recognize the impact of CS extract on patients with schizophrenia. Crocin or SAE were put into identical capsules. Each crocin capsule has 15 mg dried crocin, and each saffron capsule included 15 mg dried SAE. Vehicle-filled placebo capsules were also used [14, 15, 16]. In the case of using Crocus sativus extract in combination with the antipsychotic olanzapine, it had a beneficial effect in the case of metabolic syndrome (including hyperglycemia, triglyceridemia, decrease in HDL and cardio metabolic risk) [17, 18, 19].

Withania somnifera (WS)

There are 23 species in the Withania genus (Solanaceae), the majority of which are found in North Africa, the Canary Islands, Southern Europe, and Asia [20, 21, 22, 23, 24]. Two of the recognized species, Withania somnifera (L.) Dunal and Withania coagulans (Stocks) Dunal, are of enormous economic significance and are also primarily grown because of their broad use in natural medicine [8]. The majority of both species are grown in subtropical areas of India. W. somnifera, however, even has a bigger economic impact [25, 26]. Withania adpressa Cors. is also an endemic species in Morocco and Algeria [27], despite the fact that the morphological form and phytochemical makeup of these plants might vary depending on their geographic distribution [22, 28].

Chemestry of Withania somnifera

We describe some of the most significant withanolides isolated from Withania spp. despite the fact that they have been extensively reported by several research, taking into account their abundance, bioactive effects, and representative structures, respectively. Misra et al. [29] reported withanolide A, withanolide B, 27-hydroxy withanolide B, withanolide D, withaferin A, 16β-acetoxy-6α, 7α-epoxy-5α-hydroxy-1-oxowitha-2, 17 (20), 24-trienolide, 5, 7α-epoxy-6α, 20α-dihydroxy-1-oxowitha-2, 24- dienolide along with common steroids, β-sitosterol and sitosterol, and their glucosides in W. somnifera. Withanoside I to VII, numbered from withanoside I to 7, were isolated by Matsuda et al. [30] from the roots of W. somnifera, with class VI being the most prevalent. Similar to this, Bessalle and Lavie [31] isolated from dried leaves of W. somnifera two chlorinated withanolides, withanolide C and 4-deoxyphysalolactone [28].

The effect of *Withania somnifera* extract in schizophrenia

To get the best withanolides and aglycone concentrations devoid of impurities, Chengappa et al. employed a breveted method [32]. The WS 250 mg and a number of inactive substances were present in the tablets produced as a consequence of this method and employed in the aforementioned investigation. The trial subjects first took 2 tablets daily for a total of 500 mg throughout the first week. Additionally, they got 4 tablets, totaling 1000 mg/day, twice daily throughout the second week of the trial. According to Chengappa et al.'s findings, the PANSS scale score at the conclusion of the therapies was favorable, improving, but only in the event of an adjuvant therapy [33].

The method of extraction, the dosage of WS, the test duration of 12 weeks, and other factors were the same as those used in Chengappa and his team's experiment given by Gannon et al. At the beginning of the second week of therapy, the dosage is also increased. Their studies aims were similar to one others in wich patients with schizophrenia are monitored for symptoms of depression and anxiety. Additionally, the adjuvant WS pill therapy was good for the PANSS readings.

Discussion

It is worth mentioning that the scientific community is increasingly addressing the issue of plant extracts to obtain new treatments for neurodegenerative diseases. Although many of the studies addressed present unique processing methods of CS and WS extracts, it must be taken into ac-

count that the number of clinical researches on these extracts are very limited. In the case of depression and anxiety in the case of schizophrenia, the replications carried out by researchers are valuable and the way of working to obtain pills with WS is meticulous. However, this type of pill can be used strictly in adjunctive therapy, for depression and anxiety in the case of patients with schizophrenia.

Interesting is the abundance of scientific materials in animal models, a good example of animal models is that of Gupta and her team on anxiety induced in mice and treated with WS extract, diazepam or a combination thereof, which reduced anxiety behaviors and social isolation in mice [38]. Moreover, similar effects have been observed in traditional Chinese medicine, where local herbal preparations help patients with schizophrenia in the negative or positive symptoms they may present. This aspect is however only within adjunctive treatments [35]. Also a study carried out by the same team made a comparison only between the plant extract and antipsychotic and the participants with antipsychotic had positive and negative symptoms and a better state of health than by the herbal treatment only group [34].

The clinical study conducted by Norbala and his team is a promising one, although the data of this nature are very limited and must be viewed in the context of the study conducted by him. But what is worth mentioning is that similar results were obtained in animal models such as the mouse [36], on the plant extract Olax subscoripiodea being a shrub of African origin. Another laboratory study on mice showed the anxiolytic and antidepressant effect of the plant Maerua angolensis [37]. Thus, we can mention that the importance of plant extracts for treating depression and anxiety can be promising for finding new ways to treat depression in patients with schizophrenia.

Conclusions

Although there are notable clinical studies for the capabilities of *Crocus sativus* L. and *Withania somnifera* plant extracts, future studies are needed to form a more complete picture of the importance of plant extracts in the treatment of schizophrenia. The plant extracts could be used as adjuvant therapy for patients with schizophrenia in the context of depression and anxiety simptomsbut also the possibility of other positive or negative symptoms in schizophrenia.

References

1. Boyle M. Schizophrenia: A scientific delusion? Psychology Press, 2002.
2. Pitsikas N. *Crocus sativus* L. Extracts and Its Constituents Crocins and Safranal; Potential Candidates for Schizophrenia Treatment? Molecules. 2021 Feb 25;26(5):1237. doi: 10.3390/molecules26051237. PMID: 33669124; PMCID: PMC7956290.
3. Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. Neuron. 2000 Nov;28(2):325-34. doi: 10.1016/s0896-6273-(00)00111-2. PMID: 11144342.
4. van Os J, Kenis G, Rutten BP. The environment and schizophrenia. Nature. 2010 Nov 11;468(7321):203-12. doi: 10.1038/nature09563. PMID: 21068828.
5. Bitanihirwe BK, Woo TU. Oxidative stress in schizophrenia: an integrated approach. Neurosci Biobehav Rev. 2011 Jan;35(3):878-93. doi: 10.1016/j.neubiorev.2010.10.008. Epub 2010 Oct 23. PMID: 20974172; PMCID: PMC3021756.
6. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry. 1987 Jul;44(7):660-9. doi: 10.1001/archpsyc.1987.01800190080012. PMID: 3606332.
7. Abbott A. Schizophrenia: The drug deadlock. Nature. 2010 Nov 11;468(7321):158-9. doi: 10.1038/468158a. PMID: 21068804.
8. Field JR, Walker AG, Conn PJ. Targeting glutamate synapses in schizophrenia. Trends Mol Med. 2011 Dec;17(12):689-98. doi: 10.1016/j.molmed.2011.08.004. Epub 2011 Sep 28. PMID: 21955406; PMCID: PMC3225651.
9. Tarantilis PA, Tsoupras G, Polissiou M. Determination of saffron (Crocus sativus L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. J Chromatogr A. 1995 May 5;699(1-2):107-18. doi: 10.1016/0021-9673(95)00044-n. PMID: 7757208.
10. Liakopoulou-Kyriakides M, Kyriakidis D. *Crocus Sativus*-biological active constituents. Stud. Nat. Prod. Chem. 2002;16:293–312
11. Zheng CJ, Li L, Ma WH, Han T, Qin LP. Chemical constituents and bioactivities of the liposoluble fraction from different medicinal parts of Crocus sativus. Pharm Biol. 2011 Jul;49(7):756-63. doi: 10.3109/13880209.2010.547206. PMID: 21639689.
12. Karimi GR, Hosseinzadeh H, Khaleghpanah P. Study of antidepressant effect of aqueous and ethanol extract of Crocus sativus in mice. Iran J Basic Med Sci. 4;2001:11–15.
13. Noorbala AA, Akhondzadeh S, Tahmacebi-Pour N, Jamshidi AH. Hydro-alcoholic extract of Crocus sativus L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. J Ethnopharmacol. 2005 Feb 28;97(2):281-4. doi:

10.1016/j.jep.2004.11.004. Epub 2005 Jan 6. PMID: 15707766.

14. Fadai F, Mousavi B, Ashtari Z, Ali beigi N, Farhang S, Hashempour S, Shahhamzei N, Bathaie SZ. Saffron aqueous extract prevents metabolic syndrome in patients with schizophrenia on olanzapine treatment: a randomized triple blind placebo controlled study. Pharmacopsychiatry. 2014 Jul;47(4-5):156-61. doi: 10.1055/s-0034-1382001. Epub 2014 Jun 23. PMID: 24955550.
15. Shirali S, Bathayi SZ, Nakhjavani N, Ashoori MR. Effects of saffron (Crocus Sativus L.) aqueous extract on serum biochemical factors in streptozotocin-induced diabetic rats. Iranian Journal of Medicinal and Aromatic Plants Research. 2012;28(2):293-308. doi: 10.22092/ijmapr.2012.3045.
16. Shirali S, Zahra Bathaie S, Nakhjavani M. Effect of crocin on the insulin resistance and lipid profile of streptozotocin-induced diabetic rats. Phytother Res. 2013 Jul;27(7):1042-7. doi: 10.1002/ptr.4836. Epub 2012 Sep 5. PMID: 22948795.
17. DE Hert M, Schreurs V, Vancampfort D, VAN Winkel R. Metabolic syndrome in people with schizophrenia: a review. World Psychiatry. 2009 Feb;8(1):15-22. doi: 10.1002/j.2051-5545.2009.tb00199.x. Erratum in: World Psychiatry. 2011 Feb;10(1):78. PMID: 19293950; PMCID: PMC2656262.
18. Goff DC, Sullivan LM, McEvoy JP, Meyer JM, Nasrallah HA, Daumit GL, Lamberti S, D'Agostino RB, Stroup TS, Davis S, Lieberman JA. A comparison of ten-year cardiac risk estimates in schizophrenia patients from the CATIE study and matched controls. Schizophr Res. 2005 Dec 1;80(1):45-53. doi: 10.1016/j.schres.2005.08.010. Epub 2005 Sep 28. PMID: 16198088.
19. Meyer J, Koro CE, L'Italien GJ. The metabolic syndrome and schizophrenia: a review. Int Rev Psychiatry. 2005 Jun;17(3):173-80. doi: 10.1080/09540260500071798. PMID: 16194788.
20. Kulkarni SK, Dhir A. Withania somnifera: an Indian ginseng. Prog Neuropsychopharmacol Biol Psychiatry. 2008 Jul 1;32(5):1093-105. doi: 10.1016/j.pnpbp.2007.09.011. Epub 2007 Sep 21. PMID: 17959291.
21. Gifri AN. Studies in the Flora of Yemen. II: New records for the Flora of Aden. Candollea. 1992;47:613–619.
22. Kumar A, Kaul MK, Bhan MK, Khanna PK, Suri KA. Morphological and chemical variation in 25 collections of the Indian medicinal plant, Withania somnifera (L.) Dunal (Solanaceae). Genet. Resour. Crop Evol. 2007;54:655–660.
23. Rajeswara Rao BR, Rajput DK, Nagaraju G, Adinarayana G. Opportunities and challenges in the cultivation of Ashwagandha {*Withania somnifera* (L.) DUNAL} J. Pharmacogn. 2012;3:88–91.
24. Udayakumar R, Kasthurirengan S, Mariashibu TS, Sudhakar B, Ganapathi A, Kim EJ, Jang KM, Choi CW, Kim SC, Analysis of genetic variation among populations of Withania somnifera (L.) in South India based on RAPD markers. European J. Med. Plants. 2013;3:266–280.
25. Mir BA, Koul S, Kumar A, Kaul MK, Soodan AS, Raina SN (2013) Assessment and characterization of genetic diversity in *Withania somnifera* (L.) Dunal using RAPD and AFLP markers. Afr J Biotechnol 2011;10(66):14746–14756.
26. Umadevi M, Rajeswari R, Rahale CS, Selvavenkadesh S, Pushpa R, Kumar KPS, Bhowmik D. Traditional and Medicinal Uses of Withania Somnifera. Pharma Innov. 2012;1:102.
27. Saroya AS. Contemporary phytomedicines. CRC Press. 2017.
28. Sharifi-Rad J, Quispe C, Ayatollahi SA, Kobarfard F, Staniak M, Stępień A, Czopek K, Sen S, Acharya K, Matthews KR, Sener B, Prasad Devkota H. Chemical Composition, Biological Activity, and Health-Promoting Effects of *Withania somnifera* for Pharma Food Industry Applications. J. Food Qual. 2021;7. doi: 10.1155/2021/8985179.
29. Misra L, Mishra P, Pandey A, Sangwan RS, Sangwan NS, Tuli R. Withanolides from Withania somnifera roots. Phytochemistry. 2008 Feb;69(4):1000-4. doi: 10.1016/j.phytochem.2007.10.024. Epub 2007 Dec 3. PMID: 18061221.
30. Matsuda H, Murakami T, Kishi A, Yoshikawa M. Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian Withania somnifera DUNAL. and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. Bioorg Med Chem. 2001 Jun;9(6):1499-507. doi: 10.1016/s0968-0896(01)00024-4. PMID: 11408168.
31. Bessalle R, Lavie D. Withanolide C, a chlorinated withanolide from *Withania somnifera*. Phytochemistry. 1992;31:3648–3651.
32. Ghosal S. Withania somnifera composition, method for obtaining same and pharmaceutical, nutritional and personal care formulations thereof. 2008.
33. Gannon JM, Brar J, Rai A, Chengappa KNR. Effects of a standardized extract of Withania somnifera (Ashwagandha) on depression and anxiety symptoms in persons with schizophrenia participating in a randomized,

placebo-controlled clinical trial. Ann Clin Psychiatry. 2019 May;31(2):123-129. PMID: 31046033.

34. Zhang LD, Tang YH, Zhu WB, Xu SH. Comparative study of schizophrenia treatment with electroacupuncture, herbs and chlorpromazine. Chin Med J (Engl). 1987 Feb;100(2):152-7. PMID: 3109844.
35. Zhang Liang Dong, Zhou Gang, Jin Zong Yu. Biochemical study on TCM Treatment of Schizophrenia Based on SyndromeTyping. Journal of Traditional Chinese Medicine. 1997; 38(3):173-75.
36. Adeoluwa OA, Aderibigbe AO, Bakre AG. Evaluation of Antidepressant-like Effect of Olax Subscorpioidea Oliv. (Olacaceae) Extract in Mice. Drug Res (Stuttg). 2015 Jun;65(6):306-11. doi: 10.1055/s-0034-1382010. Epub 2014 Jul 3. PMID: 24992494.
37. Benneh CK, Biney RP, Adongo DW, Mante PK, Ampadu FA, Tandoh A, Jato J, Woode E. Anxiolytic and Antidepressant Effects of *Maerua angolensis* DC. Stem Bark Extract in Mice. Depress Res Treat. 2018 Sep 9;2018:1537371. doi: 10.1155/2018/1537371. PMID: 30271633; PMCID: PMC6151235.
38. Gupta GL, Rana AC. Protective effect of Withania somnifera dunal root extract against protracted social isolation induced behavior in rats. Indian J Physiol Pharmacol. 2007 Oct-Dec;51(4):345-53. PMID: 18476388.

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3841-3848
doi: 10.25083/rbl/28.1/3841.3848

Received for publication, July, 27, 2023
Accepted, August, 14, 2023

Original article

Investigation of the antitumoral activity of *Arthrospira platensis* (*Spirulina platensis*) in mice

HÜSEYIN TEMEL[1], *SIBEL BAYIL OĞUZKAN[2], İZZETTIN GÜLER[3], ÖMER ERONAT[4], MEHMET ÖZASLAN[5]

[1-5] Faculty of Biology, University of Gaziantep, Turkey
[2] School of Health Sciences, University of Gaziantep, Turkey
[3] İslahiye M.Y.O. Medical Services And Techniques, University of Gaziantep, Turkey
[4] Faculty Of Medicine Department Of Surgical Medical Sciences, University of Gaziantep, Turkey

Abstract

In this study, the anti-tumoral effect of *S. platensis* was investigated in Balb-c mice with EAT (Ehrlich Acid Tumor). *S. platensis* was applied in the form of 200 mg/kg and 800 mg/kg concentrate. Blood taken from mice was separated into their serum and TAL (Total Antioxidant Level), TOL (Total Oxidant Level), ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) parameters were studied. Kidney, stomach, small intestine and large intestine tissues of the sacrificed subjects were removed and evaluated pathologically. As a result, when the biochemical values of *S. platensis* activity and control groups were compared statistically there was no statistically significant difference, excepting TOL. Also, in the pathological evaluation, the spread of the tumor in organs such as the large intestine and stomach was statistically significantly different.

Keywords

S. platensis, EAT, TAL, TOL, ALT, AST

To cite this article: TEMEL H. Investigation of the antitumoral activity of *Arthrospira platensis* (*Spirulina platensis*) in mice. *Rom Biotechnol Lett.* 2023; 28(1): 3841-3848 DOI: 10.25083/rbl/28.1/3841.3848

*Corresponding author: Sibel Bayıl Oğuzkan. email: bayil@gantep.edu.tr

Introduction

Microalgae are phototrophic microorganisms with anaerobic metabolism [1] algae; Since they are the primary producers of watery environments, they form an organic food source for heterotrophic organisms, and they also provide oxygen to the environment by photosynthesis. S. platensis is a prokaryotic organism consisting of filamentous (helical), a few millimeters long, 3-12 μm thick cylindrical cells from the Cyanophyceae (Blue-green Algae) class [2]. S. platensis is an economically important algae containing 60-70% protein. It is also very rich in terms of vitamins (provitamin A, B12) and minerals (Fe, Ca, Mg) and organic coloring substances (green chlorophyll, phycocyanin, carotenoids). Due to the absence of cellulose membrane, it is easily digestible and has no toxic effects. S. platensis is the most widely used cyanobacteria and has been extensively studied in the medical and food industries [5]. Due to its high protein, minerals and vitamins, it can be used as a supplement in the treatment of many diseases [3]. In addition to its rich nutritional content, it also exhibits anti-inflammatory, anti-oxidative stress and immune-enhancing properties [6] and [7]. In fact, several studies have concluded that dietary Spirulina is helpful in the treatment and prevention of diabetes, diabetic nephropathy, hypercholesterolemia, and cancer [8] and [9]. Spirulina is widely researched in the pharmacological field due to its anti-inflammatory, antioxidant and anticancer effects [10] and [11]. It has been reported that Spirulina has an effect on the humoral and cellular immune system, stimulates lymphocytes in the blood [12], and increases the production of IgM antibodies in the spleen [13].

Hayashi & al. (1994 [23] reported that Spirulina protected from viral infections in cultured human and monkey cells. The antitumoral activity of marine algae was first studied by Nakazawa in aqueous extracts. It has been reported that the polysaccharide content of aqueous extracts is associated with antitumor activity [14]. Spirulina contains water-soluble photosynthetic protein-pigment complex with high antioxidant effect such as C-phycocyanin. In our study, the antitumoral activity of spirulinas produced in shössler medium in summer season conditions in Yalova University Armutlu Vocational School was evaluated.

Materials and methods

Balb – c Procurement of mice

In our study, 46 male Balb-c mice weighing 20-35 g were used. Experimental animals were provided by Gaziantep University Experimental Animals Research Center and were fed with standard pellet feed and water, providing a 12-hour day/night period at 25 ºC room temperature. After the approval of our study by Gaziantep University Experimental Animals Ethics Committee dated 24/04/2019 and numbered 95, all of the studies on animals were carried out at Gaziantep University Experimental Animals Research Center

EAT Tumor model

The EAT cell was obtained from Istanbul University Aziz Sancar Experimental Medicine Research Institute Laboratory Animals Department and brought to our laboratory by maintaining the cold chain. The EAT cells obtained from the tumor-formed stock animal were taken from the mouse and transferred to the mice in the Spirulina Treatment (200 mg/kg), Spirulina Treatment (800 mg/kg), 5-fluorouracil (20 mg/kg) and Control (EAT+H_2O) groups to be used in the study. .05 ml was injected i.p.

In the study, 7 groups were formed, each of which included 6 animals.

Assessment of blood samples

At the end of the application, cardiac blood was collected from all animals with a heparinized syringe. Serum was obtained from the blood taken, and TOL, TAL, ALT and AST levels were studied in order to determine the oxidant level in the serum. TAL and TOL levels were studied using the Rel Assay Diagnostics-TAL-TOL Assay Kit [21] and [22]. ALT and AST parameters were also studied using a kit from serum obtained to determine the blood value of liver damage.

The 7 groups formed in the study

	Groups	Way of delivery	Amount of delivery	Time
1	Spirulina platensis (200 mg/kg)	hesitation	0,03 ml (in distilled water)	10 days
2	Spirulina platensis (800 mg/kg)	hesitation	0,03 ml (with distilled water)	10 days
3	Spirulina platensis (200 mg/kg) + EAT	hesitation	0,03 ml (with distilled water)	1. day EAT- from 7 to 4 days Spirulina platensis
4	Spirulina platensis (800 mg/kg) + EAT	hesitation	0,03 ml (with distilled water)	1. gün EAT- 7 days from day 4Spirulina platensis
5	5-fluorouracil (200 mg/kg) + EAT	i.p	20 mg/kg	1. day EAT- 7 days from day 4 5-fluorouracil
6	Control d(H_2O) + EAT	hesitation	0,03 ml	1. day EAT- 7 days from day 4 distilled water
7	Control d(H_2O)	hesitation	0,03 ml	10 days

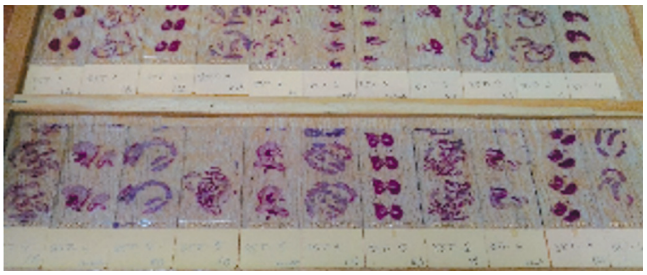


Figure 1: A frame from an image of tissues prepared for examination under a light microscope.

Histopathology

After undergoing routine tissue follow-up procedures with ethanol and xylene solutions at the Laboratory of the Department of Pathology of Gaziantep University Research Hospital, the blocking process was performed in paraffin. The prepared blocks were divided into sections with a thickness of 5µm and taken on lams as picture 1. Subsequently, the xylene evaporation process and the hydration process with ethanol were performed.

At the end of the study, small intestine, large intestine, stomach and kidney tissues were removed from the animals and placed in 10% formaldehyde. Then, the tissue was followed and the sections were taken and the preparation was prepared by staining with the "hematoxylin-eosin" method. The prepared preparations were evaluated by the pathologist with the help of a light microscope to determine whether there was tumor development in the tissues and the metastasis status of the tumor. Histopathological studies were carried out in Gaziantep University Research Hospital Pathology Department Laboratory.

Statistical Analysis

Statistical analyzes were performed according to the SPSS 21 program. Results were expressed as standard deviation or as a percentage. The t-test was used to measure the effect values on the groups, and the ANOVA test was used to reveal the differences between the groups. The p values of all statistical tests were two-sided and $p<0.05$ was considered statistically significant.

Results

When the analysis results are examined, the differences in biochemical parameters between the tumor-free groups given different doses of spirulina (200 mg/kg and 800 mg/kg) are as shown in Table 1. When the values were compared, it was determined that there was no statistically significant difference. ($p>0.05$)

When the groups given only different doses of spirulina (200 mg/kg and 800 mg/kg) were compared with the control group (Table 2), it was determined that there was no statistically significant difference in terms of TAL, TOL, OSI, ALT, AST values. ($p>0.05$)

It was determined that there was no statistically significant difference between TAL, ALT, AST ($p>0.05$) values in the treatment groups given different doses of EAT + spirulina (200 mg/kg and 800 mg/kg) (Table 3). However, it was determined that there was a statistically significant difference between these two groups in terms of TOL values (Table 3) ($p<0.05$).

There was no statistically significant difference in terms of TAL, TOL, OSI, ALT, AST values (Table 4) between the tumor and the treatment groups given different doses of spirulina (200 mg/kg and 800 mg/kg) and the tumor animals given 5-fluorouracil. determined ($p>0.05$).

When EAT+ Spirulina 200 mg/kg, EAT + Spirulina 800 mg/kg, EAT+ 5-fluorouracil and tumor-free healthy animals were compared, it was determined that there was no significant difference ($p>0.05$).

Histopathological Examination of EAT

At the end of the experiment, animals in all groups were sacrificed and the stomach, kidney, large intestine and small intestine tissues were removed by removing the ambuloc. All tissues were kept in 10% formaldehyde for 24 hours, then passed through different concentrations of alcohols and xylol and fixed within 24 hours. Then, tissue sections of 4 micron thickness were taken from the tissue samples prepared with paraffin blocks and deparaffinization process was applied. Afterwards, the samples stained with hematoxylin were cleared in xylol and evaluated under Nikon brand light microscope.

Preparations prepared from tissues were examined histopathologically. At the end of these examinations, tumor invasion was found in the kidney, stomach, large intestine and small intestine tissues in the groups given the tumor, and the tumor was evaluated by scoring method. The percentage of presence in the organs is accepted as 0, since there is no presence of tumor in the groups in which no tumors are formed. The extent of spread in organs of all groups is shown in Table 6.

According to the results of our analysis, the spread of EAT on the large intestine and stomach tissue was found to be statistically significant. ($p<0.05$). As a result of the Dunnett test, which was performed to determine which group the EAT spreads were in favor of, it was determined that the EAT spreads in the animals treated with Treatment and 5-fluorouracil were significantly lower than the control group (EAT+H2O).

The spread of EAT on the small intestine and kidney tissues was not statistically significant. ($p>0.05$). However, the

Table 1 - TAL– TOL, OSI and ALT – AST values of the groups given only spirulina (1st and 2nd Group)

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidatif stres index	ALT (U/L)	AST (U/L)
1. Group	0,47 ± 0,37	0,0132 ± 0	3,98 ± 2,03	43,83 ± 10,83	389,67 ± 128,9
2. Group	0,26 ± 0,07	0,0116 ± 0	4,66 ± 1,09	48,25 ± 23,13	458,25 ± 299,3
p value	0,321	0,172	0,562	0,69	0,626

Table 2 - TAL – TOL, OSI and ALT – AST values of only spirulina given (1st and 2nd Group) and Control (6th Group) groups

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres ndex	ALT (U/L)	AST (U/L)
1. Group	0,47 ± 0,37	0,0132 ± 0	3,98 ± 2,03	43,83 ± 10,83	389,67 ± 128,9
2. Group	0,26 ± 0,07	0,0116 ± 0	4,66 ± 1,09	48,25 ± 23,13	458,25 ± 299,3
6. Group	0,47 ±0,19	0,01 ± 0,01	2,06 ± 1,58	46,5 ± 10,21	412,25 ± 186,95
p value	0,407	0,456	0,066	0,888	0,867

Table 3- Group 3 and 4 TAL – TOL, OSI and ALT – AST values

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres index	ALT (U/L)	AST (U/L)
3. Grup	0,655 ± 0,75	0,0132 ± 0,001	3,47 ± 1,77	36,67 ± 14,39	449,83 ± 204,42
4. Grup	0,3131 ± 0,08	0,0116 ±0	3,88 ± 0,093	38,2 ± 6,72	473,2 ± 73
p değeri	0,34	0,043	0,656	0,832	0,815

Table 4 - Group 3,4 and 5 TAL – TOL, OSI and ALT – AST values

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres index	ALT (U/L)	AST (U/L)
3. Group	0,655 ± 075	0,0132 ± 0	3,468 ± 1,77	36,667 ±14,4	449,83 ± 204,4
4. Group	0,313 ± 0,08	0,0116 ± 0	3,876 ± 0,93	38,2 ± 6,72	473,2 ±73
5. Group	0,377 ± 0,23	0,0138 ± 0	4,743 ±2,47	44,25 ± 13,9	468 ± 61,42
p value	0,502	0,061	0,55	0,627	0,96

Table 5: Group 3, 4.5 and 7 TAL – TOL, OSI and ALT – AST values

Groups	TAL (mmol/L)	TOL (mmol/L)	Oxidative stres index	ALT (U/L)	AST (U/L)
3. Group	0,655 ± 0,75	0,013 ± 0	3,4681 ± 1,77	36,67 ± 14,4	449,83 ± 204
4. Group	0,313 ± 0,08	0,012 ± 0	3,8758 ± 0,93	38,2 ± 6,72	473,2 ± 73
5. Group	0,377 ± 0,23	0,014 ± 0	4,7427 ± 2,47	44,25 ± 13,9	468 ± 61,42
7. Group	0,36 ± 0,06	0,01 ± 0	4,07 ± 0,3	33,5 ± 3	422 ± 62,76
p value	0,587	0,081	0,67	0,578	0,938

Table 6 - EAT tumor spread in the small intestine, large intestine, stomach, and kidneys

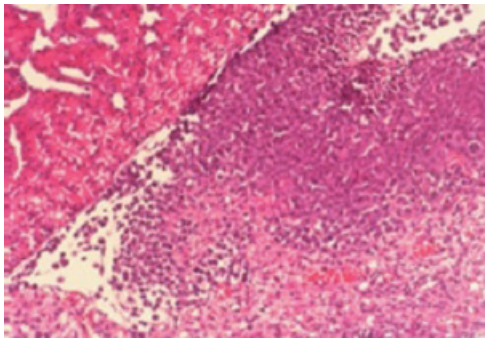
Name of group	Large intestine		Small intestine		Stomach		Kidney	
	Mean Organ Spread %	Result of pathology	Mean Organ Spread %	Result of pathology	Mean Organ Spread%	Result of pathology	Mean Organ Spread%	Result of Pathology
Spirulina (200 mg/kg)	0	(-)	0	(-)	0	(-)	0	(-)
Spirulina(800 mg/kg)	0	(-)	0	(-)	0	(-)	0	(-)
EAT + Spirulina (200 mg/kg)	7	(+)	8	(+)	21	(+)	13	(+)
EAT + Spirulina (800 mg/kg)	10	(+)	13	(+)	30	(+)	19	(+)
EAT + 5-Fluorouracil	6	(+)	8	(+)	14	(+)	14	(+)
Control (EAT+H_2O)	11	(+)	14	(+)	38	(+)	23	(+)
Control (H_2O)	0	(-)	0	(-)	0	(-)	0	(-)

Table 7 - Evaluation of EAT in Groups with Tumor (Group 3: EAT+ Spirulina 200 mg/kg, Group 4: EAT+Spirulina 800 mg/kg, Group 5 EAT+ 5-fluorouracil and Group 6: EAT+ H_2O)

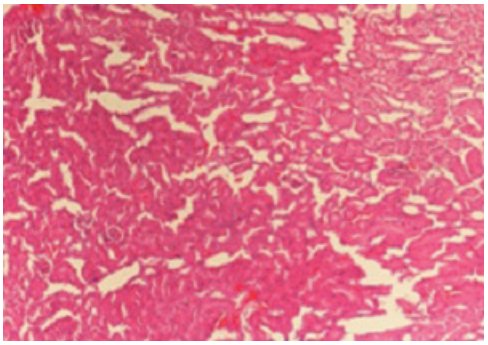
Group	Large intestine	Small intestine	Stomach	Kidney
	Organ involvement average %	Organ involvement average %	Organ involvement average %	Organ involvement average %
3. Group	6,67 ± 2,58	8,33 ± 4,08	20,83 ± 15,94	13,33 ± 5,16
4. Group	11 ± 4,18	14 ± 8,94	30 ± 6,12	19 ± 10,25
5. Group	6,25 ± 2,50	7,50 ± 2,89	13,75 ± 4,79	13,75 ± 6,29
6. Group	11,25 ± 2,50	13,75 ± 17,5	37,50 ± 10,41	22,50 ± 10,41
p	0,039	0,615	0,034	0,304

difference between the mean spreads of EAT in the kidney tissue was found to be statistically significant. For this reason, Dunnett's test was performed to determine which group favored the spread of EAT in the kidney tissue. As a result of the test, it was determined that the EAT spreads in the animals treated with treatment and 5-fluorouracil were significantly lower than the control group (EAT+H2O).

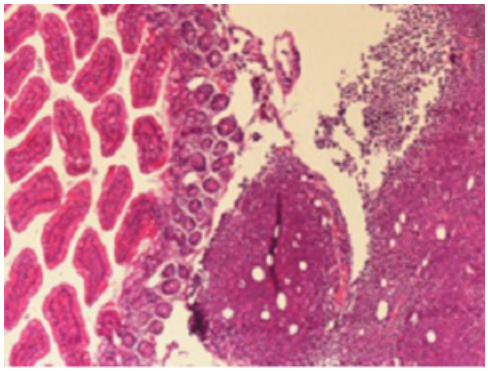
Kidney, small intestine, large intestine and stomach tissue sample sections of EAT and Control groups in Table 6 and Table 7 (H&E X 100)



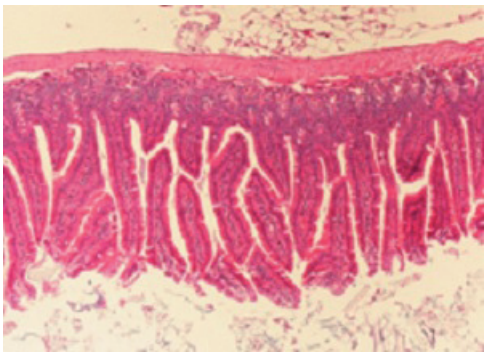
Picture 1: Kidney (with EAT)



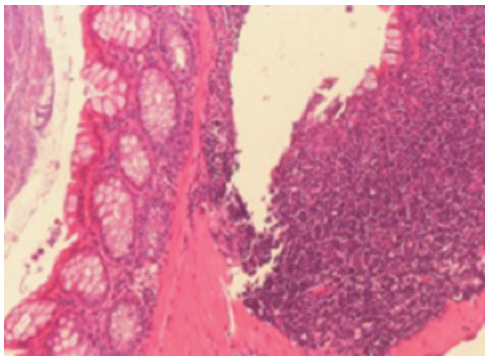
Picture 2: Kidney (Control)



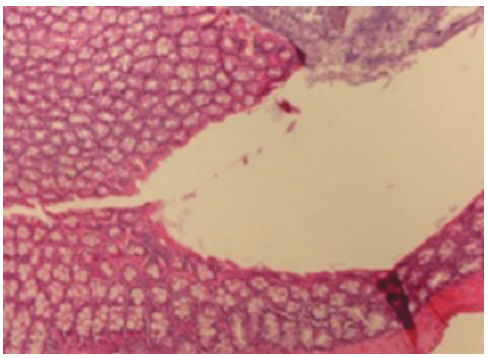
Picture 3:Small intestine (with EAT)



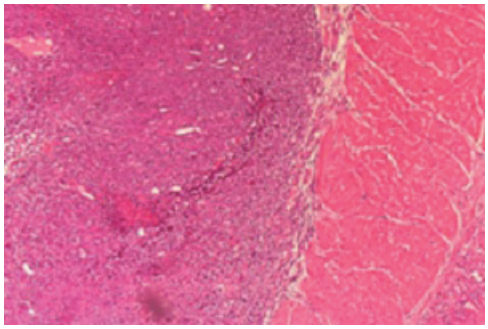
Picture 4: Small intestine (Control)



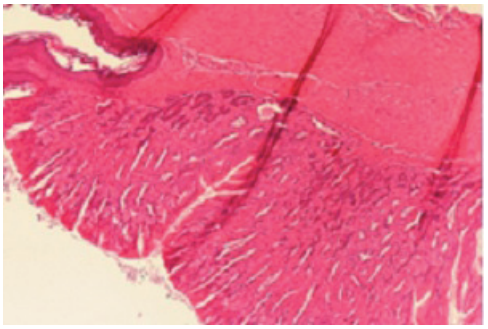
Picture 5: Large intestine (with EAT)



Picture 6: Large intestine (Control)



Picture 7: Stomach (with EAT)



Picture 8: Stomach (Control)

Discussion

Microalgae have been one of the main food and livelihood sources of people for many years in many countries due to their organic growth and development in aquatic environments (seas, lakes, fresh waters) and their rich food content [20] and J. Subhashini & al., 2004 [11]. While it is used as a nutritional supplement for humans, it is also actively used as animal feed in the poultry industry and aquaculture [20]. The use of microalgae in treatment began with the history of humanity. Thousands of years ago, people learned the therapeutic aspect of algae and benefited from them to lead a healthy life. As a result of studies on algae used in pharmacy and medicine, spirulina's enhancing effects on antimicrobial, cytotoxic, anti-mitogenic, anticancer and anti-tumoral activities were mentioned [1]. Noda et al. They examined the antitumor activity of polysaccharide and lipid parts of 24 different algae species against EAT and determined that some species showed significant effects.

Research on compounds with antitumor effects from algae continues [16] and [17]. In this study, EAT modeling of *S. Plantesis* algae produced in Yalova University Algae Production Unit was created and its anti-tumoral activity and biochemical effects were investigated in vivo on Balb-c mice. Barakat et al. In a study conducted in 2015, they determined the minimum and maximum dose (200 mg/kg, 800 mg/kg) for EAT to be similar to human breast tumor and for the anticancer activities of Spirulina platensis. When S. platensis or anti-tumoral activity studies in different species were examined, it was reported that spirulina was given by gavage method. When the group given 5-Fluorouracil was compared with the control group, it was observed that the tumor volume decreased. No significant decrease in tumor volume was observed in the groups given 200 mg/kg and 800 mg/kg S. platensis compared to the control group. has been observed to decrease significantly [18]. In our study, when the 5-Fluorouracil, *S.platensis*, 200 mg/kg and 800 mg/kg groups were compared, the EAT spread of the 5-Fluorouracil group and the 200 mg/kg S.platensis group gave significant results in the stomach and large intestine. The anti-tumoral activity of *S.platensis* is in parallel with this study.

Jiang et al. The effect of phycocyanin obtained from Spirulina on tumor progression and metastasis potential on rats with colon cancer by DMH was investigated. As a result of the study, it was observed that after the rats were induced with DMH, the number and size of tumors/lesions were reduced in those treated with phycocyanin. In our study, spirulina was given as a whole without being separated into its components. Also, Jiang et al. While colon cancer was formed on rats with DMH in our study, tumor formation was achieved with EAT in our study. As a result of the histopathological study, it was observed that the spread of EAT tumor in the large intestine was statistically significantly lower in the treatment groups given spirulina 200 mg/kg. Both studies show that spirulina is effective against cancer in the large intestine.

Ouhtit & al. (2014 [19] investigated the chemical inhibitory effect and underlying mechanisms of action of Spirulina against mammary carcinogenesis on 7,12-dimethylbenz[a] anthracene (DMBA)-induced female albino rats. It has been reported that Spirulina cleared DMBA-induced rat mammary tumors, which was clearly confirmed by morphological and histological methods, and Spirulina supplementation reduced the incidence of mammary tumors from 87% to 13%. In our study, tumor formation was achieved by administering EAT, which is similar to human mammary tumors, on Balb-c mice. As a result of the histopathological examination performed in the treatment group with Spirulina, which we gave at a dose of 200 mg/kg, it was observed that the spread of EAT tumor in the large intestine and stomach decreased, and Spirulina was found to be effective against the tumor. In our previous study, the effectiveness of phycocyanins obtained from spirulina produced in Yalova University Algae unit was investigated against EAT tumor cells, and it was found that antioxidant levels were higher in the treatment groups compared to the control [24]. In our study, significant differences were found between oxidant levels in the treatment group. Again, in our study with phycocyanin, at the end of the pathological evaluations (200 mg) phycocyanin was determined to be effective against EAT in both treatment and protection groups, and in our current study, a statistical difference was found in the spread of the tumor in the large intestine and stomach organs.

In this study, in which the antioxidant and hepatoprotective effects of Spirulina platensis were evaluated in determining the in-vivo anti-tumoral activity; The biochemical parameters of the spirulina groups created to measure the value of Spirulina depending on the dosage effect on normal mice were compared and it was found that it was not statistically significant. When we compared the biochemical parameters of the control group with spirulina in different dosages, differences were observed, but it was not found statistically significant. The biochemical parameters of the treatment groups at different dosages were compared and were not found to be statistically significant, but the differences in TOL values were found to be statistically significant. Differences in biochemical values (TAL, TOL) were found between Spirulina treatment groups, 5-fluorouracil and Control groups, but it was not found to be statistically significant.

In the histopathological examination (large intestine, small intestine, stomach, kidney) of Spirulina, treatment groups, 5-fluorouracil and control groups, it was observed that there were differences in the spread of EAT in the organs.

In the histopathological examination of the group in which we gave S. platensis at a dosage of 200mg/kg, EAT spreads were found to be statistically significantly lower in the stomach and large intestine. In the kidney, it was found statistically significant when the mean spread of EAT was taken.

The fact that spirulina causes a decrease in oxidant parameters and the spread of EAT in the stomach and intestines is less than the control group shows that spirulina has an inhibitory effect on EAT spreads.

Acknowledgment

S.Plantesis used in this study was produced in Yalova University Armutlu Vocational High School Algae Production Unit by Prof. Dr. It was produced with the supervision and contribution of Betül Güroy. Thank you for your contribution to the study.

References

1. S. Aktar, G.E. Cebe, Alglerin Genel Özellikleri, Kullanım Alanları Ve Eczacılıktaki Önemi, Ege University, Faculty Of Pharmacy, Department Of Pharmaceutical Botany, 35100 Bornova-İzmir, Turkey, Ankara Ecz. Fak. Derg. 2010, V.39 (3) Pp. 237-264;
2. S. Aksay, R. Arslan, Spirulina'dan (Spirulina Platensis) Klorofil-A Ve Fikosiyanin Pigment Ekstraksiyonuna Ultrasonikasyon Süresinin Etkisi, Akademik Gıda, 2018, 16(3) 307-312, Doi: 10.24323/Akademik-Gida.475362;
3. H. Yilmaz, M. Duru, Syanobakteri Spirulina Platensis'in Besin Kimyası Ve Mikrobiyolojisi, Türk Bilimsel Derlemeler Dergisi, 2011, 4(1): 31-43;
4. M. Qureshi, A. RA; Spirulina Platensis Exposure Enhances Macrophage Phagocytic Function In Cats, Immunopharmacol Immunotoxicol, 1996, 18(3):457-63;
5. R. Koníčková, K. Vaňková, J. Vaníková, et al. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Ann 1072 Ge et al. The efficacy of Spirulina in tumor chemotherapy © Translational Cancer Research. All rights reserved. Transl Cancer Res 2019, 8(4):1065-1073;
6. I.P. Joventino, H.G. Alves, L.C. Neves, The microalga Spirulina platensis presents anti-inflammatory action as well as hypoglycemic and hypolipidemic properties in diabetic rats. J Complement Integr Med 2012, 9:Article 17;
7. C.H. Nielsen, P. Balachandran, O. Christensen, et al. Enhancement of natural killer cell activity in healthy subjects by Immulina(R), a Spirulina extract enriched for Braun-type lipoproteins, Planta Med, 2010, 76:1802-8;
8. S. Buono, A.L. Langellotti, A. Martello, et al. Functional ingredients from microalgae. Food Funct, 2014, 5:1669-85;
9. P.D. Karkos, S.C. Leong, C.D. Karkos, et al. Spirulina in clinical practice: evidence-based human applications. Evid Based Complement Alternat Med, 2011, 2011:531053;
10. V.B. Bhat, K.M. Madyastha, C-Phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro, Biochemical and Biophysical Research Communications, 2000, 275, 1, 20–25;
11. J. Subhashini, S. V. K. Mahipal, M.C. Reddy, M. M. Reddy, A. Rachamallu, P. Reddanna, Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line-K562," Biochemical Pharmacology, 2004, 68,3; 453–462;
12. M. Qureshi, J. Garlich, M. Kidd, Dietary Spirulina Platensis Enhances Humoral And Cell-Mediated Immune Functions In Chickens, Immunopharmacol Immunotoxicol, 1996, 18(3):465-76;
13. K. Hayashi, T. Hayashi, I. A. Kojima, Natural Sulfated Polysaccharide, Calcium Spirulan, Isolated From Spirulina Platensis: In Vitro And Ex Vivo Evaluation Of Anti-Herpes Simplex Virus And Anti-Human Immunodeficiency Virus Activities, Aids Res Hum Retroviruses, 1996, 12(15):1463-71.
14. E. Taskin, Z. Caki, M. Ozturk, E. Taskin, O. Kurt, Antimicrobial and antitumoral activities of marine algae, International Review of Hydrobiology, 2010, 3 (1), 37-50;
15. N. Kaba, E. Çağlak, Deniz Alglerinin İnsan Beslenmesinde Kullanılması, E.U. Journal Of Fisheries & Aquatic Sciences, 2006, Volume 23, Suppl. (1/2): 243-246:
16. H. Güner, V. Aysel, A. Sukatar, M. Ozturk, The Aegean Sea Flora, Turkey I. Cyanophyta, Chlorophyta And Phaeophyta, Turkish Journal Of Botany, 1985, 9 (2), 277-282;
17. G. Özdemir, Z. Horzum, A. Sukatar, N. U. Karabay-Yavaşoğlu, Antimicrobial Activities Ofvolatile Components And Various Extracts Of Dictyopteris Membranaceae And Cystoseirabarbata From The Coast Of Izmir, Turkey, Pharmaceutical Biology, 2016, 44, 183-188;
18. W. Barakat, S. M. Elshazly, A. A. Mahmoud, Spirulina Platensis Lacks Antitumor Effect Against Solid Ehrlich Carcinoma In Female Mice, Advances In Pharmacological Sciences, 2015;

19. A. Quhtit, F.M. Ismail, A. Azim, A. Fernando, Chemoprevention of Rat Mammary Carcinogenesis by Spirulina. American Journal of Pathology, 2013, 184 (1);
20. N. Kaba, E. Çalak, Deniz Alglerinin İnsan Beslenmesinde Kullanılması, E.Ü. Su Ürünleri Dergisi, 2006, Volume 23:243-246;
21. M. M. TARPEY, D.A. WINK, M.B. GRISHAM, Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations, Am J Physiol, Regul Integr Comp Physiol, 2004, 286(3): R431–44;
22. B. Halliwell, J. M. C. Gutteridge, Free radicals in biology and medicine. Third ed. Oxford: Oxford Science Publications, 2000, p. 617–24;
23. O. Hayashi, T. Katoh, Y. Okuwaki, Enhancement of antibody production in mice by dietary S. platensis. J. Nutr. Sci. Vitamino, 1994, 40(5):431–441;
24. S. Bayil Oğuzkan, İ. güler, G. B. kut, K. bakir, M. özaslan, Investigation of Antitumor Activity of Phycocyanin Obtained from Spirulina (Arthrospira) platensis in Mice, International journal of pharmacology, 2023, 19: 114-121.

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3849-3859
doi: 10.25083/rbl/28.1/3849.3859

Received for publication, August, 01, 2023
Accepted, August, 14, 2023

Original article

Prevalence of *Listeria* species in raw vegetables sold in Burdur province

MERT MİDAM[1], ZEKİ EROL[1], JERINA RUGJI[1], ERDİ ŞEN[1], FULYA TAŞÇI[1],*

[1] Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, 15030, Istiklal Campus, Burdur, Türkiye

Abstract

In this research, a total of 90 raw vegetables (spinach, broccoli, lettuce, parsley, arugula, purslane, garden cress, scallions, and mushrooms) were used as materials, each of the vegetables included 10 samples obtained from different producers and district bazaars in Burdur province. *Listeria* spp. was detected in 9 (10%) of a total of 90 vegetable samples. However, none of the 9 isolated *Listeria* spp. from this study is classified as *L. monocytogenes*. Antibiotic susceptibility testing by disc diffusion method showed that 100% of the isolates were susceptible to ampicillin and penicillin G while the highest resistance has been found against meropenem and erythromycin (88.88%), and trimethoprim-sulfamethoxazole (66.66%). In conclusion, raw vegetables are considered to pose a hazard to food safety and public health due to *Listeria* species contamination.

Keywords

food safety, Listeria spp., PCR, vegetables

To cite this article: MİDAM M. Prevalence of *Listeria* species in raw vegetables sold in Burdur province. *Rom Biotechnol Lett.* 2023; 28(1): 3849-3859 DOI: 10.25083/rbl/28.1/3849.3859

*Corresponding author: Fulya Taşçı. e-mail:fulyatasci@mehmetakif.edu.tr

Introduction

People's eating habits have changed as their interest in healthy living has increased, and their demand for green-leafy vegetables and ready-to-eat salads prepared from vegetables has increased. From a nutritional point of view, vegetables are important low-calorie foods rich in vitamins, minerals, antioxidants, fibers, and bioactive compounds [1]. Leafy vegetables such as lettuce, spinach, and cabbage contain nutrients that help protect against heart disease, stroke, and cancer [2]. Vegetables, besides having significant health benefits, play an important role in the transmission of foodborne pathogens such as *E. coli* O157, Norovirus, *Salmonella, Listeria*, and *Cyclospora* [2].

Listeria is a genus of bacteria that belongs to the Listeriaceae family and has 21 species [3]. *Listeria* species can survive for long periods in various conditions such as low temperature (0-4°C), low water activity (<0.9), high salt (10-40%), and a wide pH range (4.1-9.6) [4, 5]. *Listeria* spp. is widely available in various environments due to its resistance to harsh environmental conditions. In particular, *L. monocytogenes* is the most important foodborne pathogenic microorganism among the *Listeria* species [6]. *L. monocytogenes* causes listeriosis, an invasive disease leading to meningitis, septicemia, miscarriage, or infection of newborns, as well as a noninvasive disease with flu-like symptoms [7]. Susceptible populations such as adults aged 65 years and older, children under 5 years of age, individuals with weakened immune systems, and pregnant women are more likely to contract the disease [2, 6, 7]. Although there are relatively small number of the reported listeriosis outbreaks, the mortality rate after *L. monocytogenes* infection is high, reaching 20% to 30% in the U.S. [8], and it is recognized as a major public health problem [9]. Besides *L. monocytogenes*, other *Listeria* spp. species are also reported to be virulent [6]. *L. ivanovii* and *L. monocytogenes* remain the most important species that cause listeriosis in animals and humans [10]. In fact, *L. innocua* is thought to also be virulent [10].

Several studies have reported that *Listeria* species isolated from various foodstuff, animals, humans, and the environment carry virulence genes and show resistance to many antibiotics [4, 9, 11, 12, 13, 14]. Most *Listeria* species were found to be resistant to ampicillin, rifampin, penicillin G, tetracycline, clindamycin, cephalothin, and ceftriaxone [13]. The widespread and uncontrolled use of antibiotics and their presence in the environment and in foodstuffs cause an increase in bacteria and genes resistant to antibiotics, and this poses a risk for consumer health. In this perspective, monitoring of antibiotic resistance in *Listeria* species is a necessity [15].

Foodborne pathogenic microorganisms are ubiquitous in different stages of vegetables from production to consumption. Microorganisms can enter the food chain through insects, manure, water, dust, soil, decay of vegetation, and contaminate fresh food products [7, 16]. Foodborne diseases associated with fresh vegetables have been reported to increase over the past three decades. The reasons for these increases include livestock husbandry close to the vegetable production areas, the use of animal waste and waste-contaminated waters for irrigation in the fields without any treatment, the ability of microorganisms to remain on the product for long periods of time, an increase in the number of immunocompromised persons and an increase in vegetable consumption [7].

In terms of the quantity and diversity of vegetable species cultivated, Turkey ranks high among the world's countries. Vegetable production is carried out in the form of open field and greenhouse production depending on the ecological conditions. Open field vegetable growing is carried out for table consumption and industrial production in all regions of Turkey in the form of small family-owned enterprises without any protection measures in the fields [17]. Fresh raw vegetables, especially ready-to-eat salads, are consumed raw without applying treatments that provide microbial inhibition [7]. To ensure food safety, vegetables are stored in cold warehouse facilities and washed with antimicrobial containing water solutions such as chlorine in commercial applications [16, 18]. However, it is reported that chlorine has limited antimicrobial activity if used at permissible levels, and its excessive use is associated with the production of potentially toxic substances (trihalomethanes, haloacetic acids) [19]. In addition, washing leafy vegetables is not enough to destroy microorganisms. Microorganisms adhere to the surface of the leaves and enter into them. Therefore, the raw consumption of vegetables is a major cause of foodborne illness [2]. The existence of *Listeria* species, especially *L. monocytogenes* in vegetables can pose serious health risks.

Materials and Methods

Samples

Raw vegetable samples were collected between April and July 2021 by random sampling method from different producers and district bazaars in Burdur province in the Mediterranean Region of Turkey. In this research, a total of 90 raw vegetables were used as materials, 10 samples were collected for each vegetable that include spinach (*Spinacia oleracea*), broccoli (*Brassica oleracea italica*), lettuce (*Lactuca sativa*), parsley (*Petroselinum crispum*), arugula (*Eruca vesicaria*), purslane (*Portulaca oleracea*), cress (*Lepidi-*

um sativum), scallions (*Allium fistulosum*) and mushrooms (*Agaricus bisporus*). Fresh vegetable samples were placed in sterile bags and brought to the laboratory under cold chain and analyzed within 24 hours.

Detection of *Listeria* spp. in Vegetable Samples by Cultural Method

Isolation and identification of *Listeria* species in this study were done according to ISO 11290-1:2017 standard [20]. For analysis, 25 g vegetable samples were taken in a sterile stomacher bag, 225 mL of Half Fraser broth (Oxoid CM0895) was added to it and homogenized for two minutes in a stomacher device (IUL Masticator) and incubated for 25±1 hours at 30±1°C. At the end of the incubation period, 0.1 mL was taken from the culture and added to the second selective liquid enriched culture medium, Fraser broth (Oxoid CM0895), and incubated for 24±2 hours at 37±1°C. The pre-enrichment culture obtained because of the incubation was inoculated on two selective mediums. First, it was inoculated onto chromogenic *Listeria* agar base (Merck 1.00427.0500) and incubated for 48±2 hours at 37°C. Blue-green colonies that were surrounded by an opaque halo and reproduce in the medium were considered typical *L. monocytogenes* colonies, and non-opaque blue-green colonies were considered *Listeria* spp. However, some strains of *L. monocytogenes* that are exposed to stress conditions, especially acid stress, may show a very weak halo or do not form a halo at all. Oxford agar (Oxoid CM0856) was cultured as the second selective medium and incubated for 24±2 hours at 37±1°C. The formation of a blackish-green brown-black-zoned collapse-centered colony with a diameter of 2-3 mm onto Oxford selective agar was evaluated as *L. monocytogenes* colonies. Suspicious colonies reproducing on selective medium were inoculated into non-selective Tryptone Soy Agar (TSA-YE) containing 0.6% Yeast Extract and incubated for 24±3 hours at 37±1°C. Later, the suspicious *Listeria* isolates were analyzed in terms of Gram staining, catalase, oxidase reaction, β-hemolysis, typical umbrella motility in SIM medium (Oxoid CM 435), H_2S production, indole formation, carbohydrate tests (dextrose, maltose, mannitol, rhamnose, xylose, and sorbitol) [12, 21].

Confirmation of *Listeria* spp. by PCR

Genomic DNA Extraction

Genomic DNA isolation was done using the GeneJET Genomic DNA Purification Kit according to the manufacturer's instructions (Thermo Scientific, K0721).

PCR Analysis

PCR identification of *Listeria* isolates detected as suspicious by the cultural method was performed using different primer combinations derived from the *iap* gene that is specific for *Listeria* species [22, 23, 24]. In addition, the *flaA* (363 bp) gene, which is effective in the adhesion of flagella to the surface, and the *luxS* (208 bp) gene, which plays a positive role in biofilm formation, were examined. The genes used in PCR analysis and their primary sequences are given in Table 1.

PCR Amplification and Electrophoresis

Three µL of the extracted DNA was added to each of the PCR tubes. Six µL ddH_2O (sterile nuclease-free water), 0.5 µL forward primer, 0.5 µL reverse primer and 10 µL Ruby Taq Master (2x) Mix (Jena Bioscience, Germany) containing Taq polymerase, nucleotides (dATP, dCTP, dGTP, dTTP), KCl, $(NH4)_2SO_4$), $MgCl_2$, red stain, density reagent, enhancer and stabilizing additives were added to complete them to 20 µL. They were mixed by pipetting and the PCR mixture was brought to the ready-to-use state.

Listeria spp. isolates identified by bacteriological methods were tested by conventional PCR assay. For *Listeria* spp. identification, *iap* gene was amplified using primer

Table 1 - The genes and primer sequences used in PCR analysis.

Target gene	Primer sequences (5' → 3')	Product size (bp)	Reference
iap	MonoA F: CAAACTGCTAACACAGCTACT monoB R: GCACTTGAATTGCTGTTATTG	371	A. Bubert & al., 1992 [22]
iap	UnilisA F: GCTACAGCTGGGATTGCGGT Lis1B R: TTATACGCGACCGAAGCCAA	~1400	A. Bubert & al., 1997 [23]
iap	Iva1 F: CTACTCAAGCGCAAGCGGCAC Lis1B R: TTATACGCGACCGAAGCCAAC	1112	A. Bubert & al., 1999 [24]
iap	Sel1 F: TACACAAGCGGCTCCTGCTCAAC Lis1B R: TTATACGCGACCGAAGCCAAC	1099	A. Bubert & al., 1999 [24]
iap	Wel1 F: CCCTACTGCTCCAAAAGCAGCG Lis1B R: TTATACGCGACCGAAGCCAAC	1048	A. Bubert & al., 1999 [24]
iap	Ino2 F: 5-ACTAGCACTCCAGTTGTTAAAC Lis1B R: 5-TTATACGCGACCGAAGCCAAC	1017	A. Bubert & al., 1999 [24]
luxS	F: GGAAATGCCAGCGCTACACTCTTT R: ATTGCATGCAGGAACTTCTGTCGC	208	S.R. Warke & al., 2017 [26]
flaA	F: GCGCAAGAACGTTTAGCATCTGGT R: TTGAGTAGCAGCACCTGTAGCAGT	363	S.R. Warke & al., 2017 [26]

pairs UnilisA and LislB. The PCR conditions were initial denaturation at 94°C for 3 minutes, then 30 cycles of denaturation for 1 minute at 94°C, annealing at 56°C for 45 seconds, extension at 72°C for 45 seconds, and at the end of these processes, the amplicons were kept at +4ºC until the next stage [23]. The amplification of the MonoA and MonoB primer pair was performed in the conditions of initial denaturation for 4 minutes at 94ºC, then 30 cycles of denaturation for 45 seconds at 94ºC, annealing for 30 seconds at 55ºC, extension for 10 minutes at 72ºC, and at the end of these operations, the amplicons were kept at 4ºC until the next stage [22]. In the identification of other *Listeria* species, for Ino2 and Lis1B primer pairs a 45-second 30-cycle denaturation of the at 94ºC, annealing for 60 seconds at 62ºC, and a 45-second extension at 72°C were performed. For Sel1-Lis1B, Wel1-Lis1B, and Iva1-Lis 1B primer pairs, 30 cycles of denaturation for 30 seconds at 95ºC, 30 seconds of annealing at 62°C, and 90 seconds of extension at 72°C were performed [25]. For *luxS* (263 BP) and *flaA* (363 bp) pathogenicity genes, PCR conditions were an initial denaturation for 2 minutes at 94°C, then 30-second 35-cycle at 94ºC, annealing for 30 seconds at 58ºC, and extension for 7 minutes at 72ºC, and the amplicons were kept at +4ºC until the next stage [26]. After amplification, 1.5% agarose gel containing 1xTAE buffer was prepared (Prona Agarose, Biomax) and mixed with 10 mg/mL Ethidium Bromide (SNP Biotechnology). Then, DNA Marker (GeneRuler 100 bp DNA Ladder, Thermo Scientific), positive control (*L. monocytogenes* ATCC 7644), negative control (distilled water), and sample amplicons were loaded into the wells in the gel. Electrophoresis was performed for 1 hour at a current of 100 volts in the tank (Nyx Technik Voltronyx-V37, Taiwan). Then, the band formations were imaged through the UV-transilluminator (T12621D, Taiwan).

Antibiotics Susceptibility Test

Antimicrobial susceptibility tests of the isolates were carried out by disc diffusion method [27]. *Listeria* spp. isolates were incubated in TSA (Oxoid CM 131) for 24 hours at 37°C. The cultures obtained after incubation were taken with loops and suspended in sterile tubes containing 5 mL of 0.85% physiological saline solution and adjusted to 0.50 McFarland (10^8 CFU/mL) turbitide with a McFarland densitometer device (Biosan, Lithuania). Thereafter, the prepared suspension was inoculated onto Mueller Hinton agar (MHA) (Oxoid 337) containing 5% defibrinated horse blood and 20 mg/L β-NAD (Sigma-Aldrich, N6522) by streaking the sterile swab over the surface, and then the antibiotic discs were placed. The following antibiotic discs (Bioanalyse, Türkiye) were used: ampicillin (AM, 10 µg), penicillin G (P, 10 units), erythromycin (E, 15 µg), meropenem (MEM, 10 µg), and trimethoprim sulfamethoxazole (STX, 25 µg). Antibiotic discs were incubated at 35 ±1°C for 18 ±2 hours in 5% CO_2, and inhibition halos were measured. Interpretation of antibiotic susceptibility was determined in accordance with the guidelines of the European Committee on Antimicrobial Susceptibility Testing [28] for erythromycin, meropenem, trimethoprim sulfamethoxazole (*L. monocytogenes*), and the Clinical and Laboratory Standards Institute [29] for ampicillin and penicillin G (*Enterococcus* spp.). *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 25293 were used as control cultures for the disk diffusion assay. Multidrug resistant isolates were defined as those which exhibited resistance to three or more antimicrobial classes of antimicrobial tested [30].

Statistical Analyses

The statistical analyses were performed Minitab for Windows Version Release 16.1. (Minitab Inc., 2010). The chi-square test was used to assess the differences between proportions at a significance level of 0.05.

Results

Nine (10%) of the 90 samples analyzed by the cultural method were presumed to be *Listeria* spp. Later, it was confirmed by PCR analysis that 9 of 9 isolates (100%) carried

Table 2 - Prevalence of *Listeria* spp. in raw vegetables.

Samples	n	Positive *Listeria* spp. n (%)	*Listeria* spp. positive isolates n					Isolates n
			L. monocytogenes	*L. ivanovii*	*L. seeligeri*	*L. welshimeri*	*L. innocua*	
Parsley	10	1(10)	0	0	1	0	0	1
Spinach	10	2(20)	0	2	1	1	1	5
Scallion	10	4(40)	0	0	1	2	2	5
Lettuce	10	0	0	0	1	0	0	1
Purslane	10	0	0	0	1	0	0	1
Cress	10	1 (10)	0	0	1	0	0	1
Arugula	10	1 (10)	0	1	1	1	1	4
Broccoli	10	0	0	0	1	0	0	1
Mushroom	10	0	0	0	1	0	0	1
Total	90	9 (10)	0	3	9	4	4	20

n: number of samples analyzed, a: At least one or more species were isolated from one positive sample.

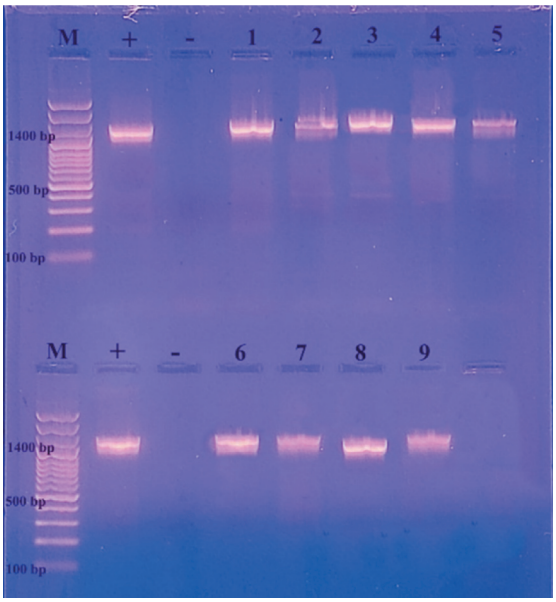


Figure 1 - Genus-specific identification of *Listeria* spp. isolates by PCR with the primer pairs UnilisA-Lis1B. Line M: molecular marker (GeneRuler 100 bp Plus DNA Ladder, Thermo, USA), Line +: positive control (*L. monocytogenes* ATTC 7644), Line -: negative control (distilled water), Lane 1: spinach 1st sample, Line 2: spinach 6th sample, Line 3: parsley, Line 4: scallion 4th sample, Line 5: scallion 5th sample, Line 6: cress, Line 7: scallion 8th sample, Line 8: scallion 10th sample, Line 9: arugula.

Listeria spp genes. Also, *Listeria* spp. was detected in 2 of spinach (20%), 1 of parsley (10%), 4 of scallions (40%), 1 of cress (10%), and 1 of arugula (10%). However, *Listeria* species were not isolated in broccoli, mushrooms, lettuce, and purslane samples. The results on the prevalence of *Listeria* spp. in the analyzed sampled vegetable are given in Table 2, and the molecular identification of *Listeria* species by specific *iap* gene primers is shown in Figures 1-2. There was no statistically significant association between the type of vegetable surveyed and the presence of *Listeria* spp. (χ^2 = 4.659; p > 0.05).

Multiplex PCR of *luxS* and *flaA* genes revealed that 4 of 9 isolates (1 spinach, 3 scallions) harbored only *flaA* gene, whilst *luxS* and *flaA* genes were detected in 3 samples (spinach, parsley, scallion) and these two genes were found to be absent in 2 vegetable samples (cress, arugula), the results being shown in Figure 3.

Antimicrobial resistance profiles of 9 isolates confirmed by PCR as *Listeria* spp. were examined. The antimicrobial resistance of *Listeria* spp. isolates is shown in Table 3. In the present study, there was no significant association between the different *Listeria* spp. isolates in terms of antibiotic resistance (χ^2 = 6.750; p > 0.05). All 9 isolates (100%) were found to be susceptible to ampicillin and penicillin G. It was determined that 8 (88.88%) of 9 isolates analyzed were resistant to meropenem and erythromycin, and 6 (66.66%) were resistant to trimethoprim sulfamethoxazole. Also, multiple antibiotic resistance profiles were determined in 5 (55.55%) of *Listeria* spp. isolates.

Discussion

The *iap* (invasion-associated protein) gene, which is common to all members of the *Listeria* genus, encodes the p60 protein and is an important marker in PCR-based analyses of *Listeria* spp. [24]. *Listeria* spp. was detected in 9 (10%) of the total 90 vegetable samples obtained from the local district bazaar of Burdur province. *L. monocytogenes* was not detected in any of the vegetable samples. However, in this study, other *Listeria* species were isolated alone or in combined forms in raw vegetable samples (Figure 2). In this study, *L. seeligeri* was detected in all vegetable samples. *L. seeligeri* is reported to survive longer as a result of using xylose derived from cellulose which is abundant in the soil [31]. Although *L. seeligeri* is reported as a hemolytic but non-pathogenic bacterium, it has rarely been reported to cause acute purulent meningitis in a healthy adult person. In addition, *L. seeligeri* is reported to be a heterogeneous species in terms of pathogenicity and may contain strains that cause life-threatening diseases in humans [32]. In this study, *L. ivanovii* was isolated in 2 scallions and 1 arugula, a total of 3 vegetable samples, also *L. welshimeri* and *L. innocua* were isolated in 4 samples including 1 spinach, 2 scallions, and 1 arugula. *L. ivanovii* is a major problem in ruminants [33] as it causes abortion, stillbirths, and encephalitis in ruminants, but, sporadic cases of listeriosis caused by *L. ivanovii* have been reported in people, especially in immunocompromised people [34, 35]. Similarly, although *L. welshimeri* and *L. innocua* are not considered dangerous to human life, they are

Table 3 - Antibiotic resistance profiles of *Listeria* spp. isolated from raw vegetable samples.

Antimicrobial agent	*Listeria* spp. isolates (n=9)	
	S n (%)	R n (%)
AM,10 µg	9 (100)	0 (0)
P, 10U	9 (100)	0 (0)
MEM, 10 µg	1 (11.11)	8 (88.88)
STX, 25 µg	3 (33.33)	6 (66.66)
E, 15 µg	1 (11.11)	8 (88.88)

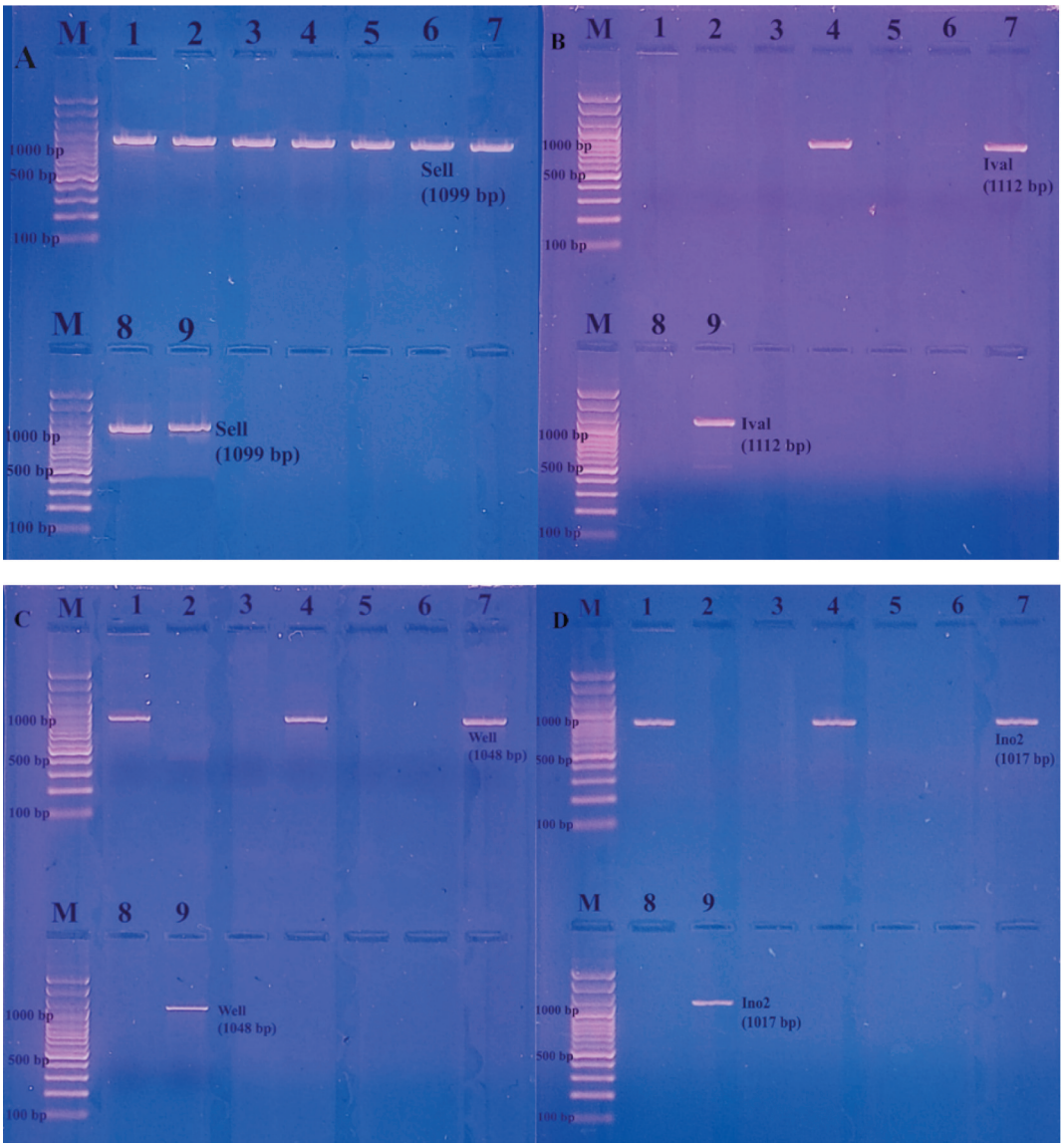


Figure 2 - Specific identification of *L. seeligeri* (A), *L. ivanovii* (B), *L. welshimeri* (C), and *L. innocua* (D) by PCR with primer pairs Sel1-Lis1B, Iva -Lis1B, Wel1-Lis1B, and Ino2-Lis1B, respectively. Line M: molecular marker (GeneRuler 100 bp Plus DNA Ladder, Thermo, USA), Lane 1: spinach 1st sample, Line 2: spinach 6th sample, Line 3: parsley, Line 4: scallion 4th sample, Line 5: scallion 5th sample, Line 6: cress, Line 7: scallion 8th sample, Line 8: scallion 10th sample, Line 9: arugula

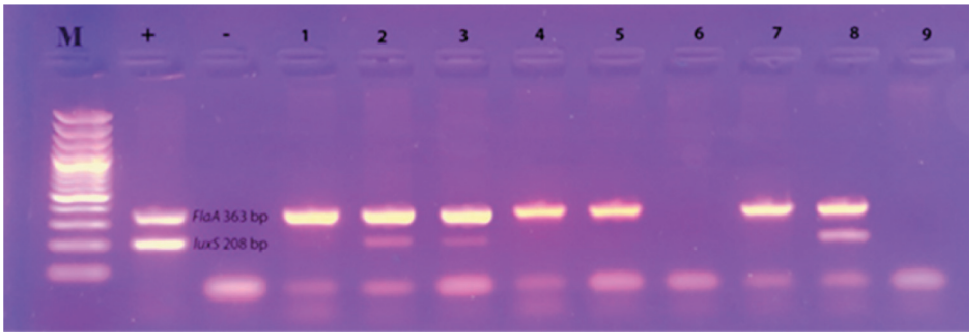


Figure 3 - Electrophorese image of luxS (208 bp) and flaA (363 bp) gene *Listeria* spp. by PCR. Line M: molecular marker (GeneRuler 100 bp Plus DNA Ladder, Thermo, USA), Line +: positive control (*L. monocytogenes* ATTC 7644), Line -: negative control (distilled water), Lane 1: spinach 1st sample, Line 2: spinach 6th sample, Line 3: parsley, Line 4: scallion 4th sample, Line 5: scallion 5th sample, Line 6: cress, Line 7: scallion 8th sample, Line 8: scallion 10th sample, Line 9: arugula

predicted to pose a potential threat sometimes to people due to reported human cases [10, 36].

Although *L. monocytogenes* was not detected in any of the raw vegetable samples analyzed in this study, *flaA* and *luxS* genes were detected in the isolated *Listeria* spp. (Figure 3). All *Listeria* species use flagella to provide mobility in *in vitro* environments, though they are mobile at 20-25°C and immobile at 37°C. Flagella are critical for both surface adhesion and subsequent biofilm formation and are associated with virulence [12, 37, 38]. *Listeria* species can cause cross-contamination in food from contacted surfaces in the environment due to their ability to adhere to surfaces and create biofilms using peritric flagella [39]. In addition, the *flaA* gene encoding the Flagellin A protein is also used in the genotypic identification of *Listeria* species [40]. The *luxS* gene encodes an enzyme called S-ribosylhomocysteinase. This enzyme catalyzes the hydrolysis of S-ribosylhomocysteine to homocysteine and 4,5-dihydroxy-2,3-pentadione (DPD) and acts as a precursor to Autoinducer-2 (AI-2) [11]. In addition to being present in many gram-positive and gram-negative bacteria, it is responsible for pathogenesis, motility, and biofilm formation [41]. Although, *L. monocytogenes* was not detected in raw vegetables is a positive result for public health, the other *Listeria* species may pose health risks.

There are very few studies conducted on the presence of *Listeria* species in vegetables in Turkey. According to these studies, S. Lee & al. (2007) [42] detected *Listeria* spp. in 15 (40.5%), and *L. monocytogenes* in 3 (8.1%) of 37 frozen peppers in Bursa province. In addition, they detected *Listeria* spp. in 2 frozen strawberries (100%) and 1 frozen Brussels sprouts (100%). *Listeria* spp. was not detected in frozen tomato, pea, and scallion samples. S.A. Aytac & al. (2010) [43] analyzed a total of 164 leafy vegetable samples (8 basils, 15 dills, 20 cresses, 16 cabbage, 12 lettuces, 19 mint, 19 parsleys, 18 purslanes, 1 radish, 20 arugulas, 14 scallions, and 2 spinach) grown in the Ankara city. While *L. monocytogenes* was not detected in radish, spinach, and scallion samples, but detected in 14 samples (3 basils, 1 dill, 1 cress, 2 cabbage, 1 lettuce, 1 mint, 2 parsleys, 1 purslane, and 2 arugulas). R. Kara & al. (2019) [44] determined *L. monocytogenes* in 1 (1.43%) of 70 fresh lettuce samples collected from grocery stores and bazaars in Afyonkarahisar province. In contrast to the research results conducted by S. Lee & al. (2007) [42], S.A. Aytac & al. (2010) [43], and R. Kara & al. (2019) [44] *L. monocytogenes* was not isolated in green vegetable samples in this study. The variations in the prevalence of *L. monocytogenes* in vegetables are reported to be related with differences in seasonal, geographical and contamination exposure levels [3]. The inability to detect *L. monocytogenes* in raw vegetable samples in this study is probably due to the proper production process of the vegetables.

Studies in various countries, also stated that vegetables were contaminated with *Listeria* species at different rates. *L. monocytogenes* was detected in 2.5% of 120 packaged lettuce samples in Australia [45], in 13 (0.34%) of 5379 samples of freshly cut vegetables sold at markets in Canada [46]. *L. monocytogenes* was not detected in ready-to-eat vegetables sold in supermarkets in Portugal [47, 48]. D.K. Sonı & al. (2014) [48] isolated *L. monocytogenes* in 20 (10%) of 200 vegetable samples and in 10 (5%) of soil samples in India. V.V. Byrne & al., 2016 [16] isolated *L. monocytogenes* in a total of 4 (3.0%) samples, including 1 (2.22%) of 45 raw vegetables and 3 (5.56%) of 54 ready-to-eat vegetables (for salads) in Brazil. A.M. Gonı & al. (2016) [49] detected *Listeria* spp. in 84 (21%) of a total of 405 vegetable samples consisting of 16 types of vegetables (green amaranth, red amaranth, coriander, water spinach, winged bean, small water pepper, basil, lettuce, mint, scallion, gotu kola (pegaga), ulam raja, cucumber, mustard flowers, watercress, water celery) collected in Malaysia. *L. monocytogenes* were found in 69 (28.28%) of cabbages, 22 (9.02%) of carrots, 57 (23.36%) of cucumbers, 48 (19.67%) of lettuce, and 48 (19.67%) of tomatoes (19.67%) in Nigeria by T.A. Ajayeoba & al. (2016) [50].

While the *L. monocytogenes* ratio was 4.2% in the total of food samples in Ireland, it was determined as 3.8% in environmental samples [51]. M. Moravkova & al. (2017) [52] detected *L. monocytogenes* in 2 (2.1%) samples out of 97 by standard culture analysis, 4 (4.1%) samples by combined culture analysis, and 1 (1.9%) sample by PCR technique among 175 green-leafy vegetable and salad samples. In West Virginia, K. Lı & al. (2017) [53] detected *Listeria* spp. in 50% of the samples after analyzing 212 fresh products including tomatoes, green peppers, cucumbers, melons, and spinach, and *L. monocytogenes* in 3.78 % of the samples following identification analyses using PCR. M. Chen & al. (2018) [54] analyzed a total of 665 mushrooms in China, 237 of which were packaged and 428 of which were not packaged, and detected *L. monocytogenes* in 141 (21.2%) of fresh mushroom samples. I. Kljujev & al. (2018) [55] reported *Listeria* spp. in 25.58% of 43 vegetable samples (16 tomatoes, 13 sweet peppers, 2 cabbages, 1 hot pepper, 1 cucumber, 5 potatoes, 4 carrots, and 1 parsley), while *L. monocytogenes* was detected only in 1 (0.43%) carrot sample in the central Serbian Region. E.O. Kyere & al. (2020) [56] detected *L. monocytogenes* in 7 (11.666%) of 60 packaged vegetable samples, while 40 non-packaged vegetables did not find *L. monocytogenes* in New Zealand. A. Samad & al. (2020) [57] determined *L. monocytogenes* in 2 (2%) of the 100 fresh

salads, but *L. monocytogenes* was not detected in 100 fresh vegetable samples in Pakistan. The findings obtained as a result of this research were found to be lower when compared with other studies [49, 55]. *L. monocytogenes* was not isolated from the samples collected in the present study, and is similar to the finding of J. Campos & al., 2013 [47], E.O. Kyere & al. (2020) [56], and A. Samad & al. (2020) [57]. *Listeria* spp. quantities in the data obtained by V.V. Byrne & al., 2016 [16], E.A. Szabo & al., 2000 [45], Health Canada, 2011 [46], D.K. Sonı & al. (2014) [48], T.A. Ajayeoba & al. (2016) [50], D. Leong & al., 2017 [51], M. Moravkova & al. (2017) [52], K. Lı & al. (2017) [53], M. Chen & al. (2018) [54], E.O. Kyere & al. (2020) [56], and A. Samad & al. (2020) [57] were found to be higher than the data presented in this study. The fact that the results of the research are different from the results of other research is thought to be caused by seasonal factors, geographical location, sample differences, raw material production and storage conditions, personnel hygiene, cross-contamination during transportation and sales, and differences in analysis methods.

The prevalence of antibiotic resistance, especially multiple antibiotic resistances, in the *Listeria* spp. is reported to be regularly rising [58]. In current study, it has been determined that *Listeria* spp. isolates are resistant to meropenem, trimethoprim-sulfamethoxazole, and erythromycin. The highest resistance has been found against meropenem and erythromycin (Table 3). Also, multidrug resistance, i.e., resistance to three or more antimicrobial classes, was observed in all *Listeria* spp. isolates. This situation suggests that it may cause public health problems for consumers. On the other hand, S. Stonsaovapak & M. Boonyaratanakornkıt (2010) [59] determined that *Listeria* spp. were resistant to penicillin, but sensitive to ampicillin, and sulfamethoxazole. L.M. Bılung & al., 2018 [13] reported that *Listeria* spp. isolated from vegetables are resistant to ampicillin, penicillin G, meropenem, and trimethoprim-sulfamethoxazole. *L. welshimeri, L. grayi, L. murrayi* and *L. innocua* cultures were reported to be sensitive to natural, semi-synthetic penicillins [14]. G. Cufaoglu & al. (2021) [60] reported the most resistant antibiotic was sulfamethoxazole (97.3%) and the less resistant antibiotic was meropenem (5.8%) in Turkey. Studies on the determination of antibiotic resistance of *Listeri*a species in vegetables and fruits are insufficient. In this study, the determination of the presence, virulence genes, and antibiotic susceptibility of *Listeria* species in the raw vegetable samples, it will contribute to other studies and epidemiological monitoring.

Conclusion

Vegetables are usually produced in insufficiently hygienic conditions and are sold wet in bazaars. In addition, it is consumed raw without any heat treatment or added as a mixture in ready-to-eat food products such as salads. Therefore, there may be serious risks in terms of the presence of *Listeria* spp. in vegetables. In this study, the fact that *L. monocytogenes* was not isolated from raw vegetables is considered as a positive result in terms of public health. However, it was concluded that detection of other *Listeria* species, *luxS* and *flaA* virulence genes, and multidrug resistance was observed in all *Listeria* spp. isolates in all of vegetables would pose a risk to public health. Also, it is considered that carrying out this research in different regions and with different vegetable species would be appropriate for determining the prevalence and virulence characteristics of *Listeria* species. To reduce contamination of vegetables at all stages from the field to the table, good agricultural practices and good hygiene practices should be carefully carried out to increase product safety in the cultivation, harvesting, classification, packaging, and distribution of fresh products.

Acknowledgements

This work was supported by the Burdur Mehmet Akif Ersoy University Scientific Research Projects Management Unit Project [0687-YL-21].

Conflicts of Interest

No potential conflict of interest was reported by the authors.

References

1. Taban BM, Halkman AK. Do leafy green vegetables and their ready-to-eat [RTE] salads carry a risk of foodborne pathogens? Anaerobe 2011 Dec;17(6):286-7. doi: 10.1016/j.anaerobe.2011.04.004. Epub 2011 Apr 29. PMID: 21549216.
2. CDC, Lettuce, other leafy greens, and food safety. Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, 2021. Retrieved from https://www.cdc.gov/foodsafety/communication/leafy-greens.html
3. Kayode AJ, Okoh AI. Incidence and genetic diversity of multi-drug resistant Listeria monocytogenes isolates recovered from fruits and vegetables in the Eastern Cape Province, South Africa. Int J Food Microbiol. 2022 Feb 16;363:109513. doi: 10.1016/j.ijfoodmicro.2021.109513. Epub 2021 Dec 25. PMID: 34971880.
4. Şanlıbaba P, Tezel BU, Cakmak GA. Prevalence and antibiotic resistance of Listeria monocytogenes isolated from ready-to-eat foods in Turkey. J Food Qual. 2018; 2018: 7693782. doi: 10.1155/2018/7693782

5. Chen M, Cheng J, Zhang J, Chen Y, Zeng H, Xue L, Lei T, Pang R, Wu S, Wu H, Zhang S, Wei X, Zhang Y, Ding Y, Wu Q. Isolation, Potential Virulence, and Population Diversity of Listeria monocytogenes From Meat and Meat Products in China. Front Microbiol. 2019 May 7;10:946. doi: 10.3389/fmicb.2019.00946. PMID: 31134008; PMCID: PMC6514097.
6. Zhao Q, Hu P, Li Q, Zhang S, Li H, Chang J, Jiang Q, Zheng Y, Li Y, Liu Z, Ren H, Lu S. Prevalence and transmission characteristics of *Listeria* species from ruminants in farm and slaughtering environments in China. Emerg Microbes Infect. 2021 Dec; 10(1):356-364. doi: 10.1080/22221751.2021.1888658. PMID: 33560938; PMCID: PMC7928038.
7. Olaimat AN, Abu Ghoush M, Al-Holy M, Abu Hilal H, Al-Nabulsi AA, Osaili TM, Ayyash M, Holley RA. Survival and growth of *Listeria monocytogenes* and *Staphylococcus aureus* in ready-to-eat Mediterranean vegetable salads: Impact of storage temperature and food matrix. Int J Food Microbiol. 2021 May 16; 346:109149. doi: 10.1016/j.ijfoodmicro.2021.109149. Epub 2021 Mar 10. PMID: 33756283.
8. FDA. Get the facts about *Listeria*, 2020, Retrieved from https://www.fda.gov/animal-veterinary/animal-health-literacy/get-facts-about-listeria.
9. Mpundu P, Muma JB, Mukubesa AN, Kainga H, Mudenda S, Bumbangi FN, Muleya W, Katemangwe P, Munyeme M. Antibiotic Resistance Patterns of *Listeria* Species Isolated from Broiler Abattoirs in Lusaka, Zambia. Antibiotics (Basel). 2022 Apr 28; 11(5): 591. doi: 10.3390/antibiotics11050591. PMID: 35625235; PMCID: PMC9137566.
10. Moura A, Disson O, Lavina M, Thouvenot P, Huang L, Leclercq A, Fredriksson-Ahomaa M, Eshwar AK, Stephan R, Lecuit M. Atypical Hemolytic *Listeria innocua* Isolates Are Virulent, albeit Less than *Listeria monocytogenes*. Infect Immun. 2019 Mar 25; 87(4):e00758-18. doi: 10.1128/IAI.00758-18. PMID: 30670551; PMCID: PMC6434133.
11. Vendeville A, Winzer K, Heurlier K, Tang CM, Hardie KR. Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. Nat Rev Microbiol. 2005 May; 3(5): 383-96. doi: 10.1038/nrmicro1146. PMID: 15864263.
12. Liu D, Lawrence ML, Ainsworth AJ, Austin FW. Genotypic Identification. In: Liu D, (Ed), *Handbook of Listeria monocytogenes* (1st edition) (pp 169-202). Boca Raton: CRC Press, Taylor & Francis Group, 2008.
13. Bilung LM, Sin Chai L, Tahar AS, Ted CK, Apun K. Prevalence, Genetic Heterogeneity, and Antibiotic Resistance Profile of *Listeria* spp. and *Listeria monocytogenes* at Farm Level: A Highlight of ERIC- and BOX-PCR to Reveal Genetic Diversity. Biomed Res Int. 2018 Jul 3;2018:3067494. doi: 10.1155/2018/3067494. PMID: 30065935; PMCID: PMC6051282.
14. Vygovska LM. Determination *Listeria* spp. (*L. welshimeri, L. grayi, L. murrayi, L. innocua*) sensitivity to antibiotics. JVMBBS 2018; 4: 33-36. UDC 619:579.869.1:615.281:615.33
15. Panera-Martínez S, Rodríguez-Melc´on C, Serrano-Gal´an V, Alonso-Calleja C, Capita R. Prevalence, quantification and antibiotic resistance of *Listeria monocytogenes* in poultry preparations. Food Control 2022; 135: 108608. doi: 10.1016/j.foodcont.2021.108608
16. Byrne VV, Hofer E, Vallim DC, de Castro Almeida RC. Occurrence and antimicrobial resistance patterns of *Listeria monocytogenes* isolated from vegetables. Braz J Microbiol. 2016 Apr-Jun;47(2):438-43. doi: 10.1016/j.bjm.2015.11.033. Epub 2016 Mar 2. PMID: 26991279; PMCID: PMC4874581.
17. Duman İ, Tüzel Y, Appelman DJ. Vegetable type and variety preference in Turkey, Ege Univ Ziraat Fak Derg. 2020; Special Issue: 169-178. doi: 10.20289/zfdergi.837441
18. Raffo A, Paoletti F. Fresh-cut vegetables processing: environmental sustainability and food safety issues in a comprehensive perspective. Front Sustain Food Syst. 2022; 5: 681459. doi: 10.3389/fsufs.2021.681459
19. Malka SK, Park MH. Fresh produce safety and quality: chlorine dioxide's role. Front Plant Sci. 2022; 12: 775629. doi: 10.3389/fpls.2021.775629
20. ISO. International Organization for Standardization, EN ISO 11290-1:2017. Microbiology of the food chain-Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp., 2017. Part 1: Detection method.
21. Hitchins AD, Jinneman K, Chen Y. BAM Chapter 10: Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods. In: Bacteriological Analytical Manual (BAM), 2017. Retrieved from https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration
22. Bubert A, Köhler S, Goebel W. The homologous and heterologous regions within the iap gene allow genus- and species-specific identification of *Listeria* spp. by polymerase chain reaction. Appl Environ Microbiol. 1992; 58(8): 2625-2632. doi: 10.1128/aem.58.8.2625-2632.1992

23. Bubert A, Riebe J, Schnitzler N, Schönberg A, Goebel W, Schubert P. Isolation of catalase-negative *Listeria monocytogenes* strains from listeriosis patients and their rapid identification by anti-p60 antibodies and/or PCR. J Clin Microbiol. 1997; 35(1): 179-83. doi: 10.1128/jcm.35.1.179-183.1997
24. Bubert A, Hein I, Rauch M, Lehner A, Yoon B, Goebel W, Wagner M. Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. Appl Environ Microbiol. 1999; 65(10): 4688-4692. doi: 10.1128/AEM.65.10.4688-4692.1999
25. Shantha SM, Gopal S. Prevalence of *Listeria* species in environment and milk samples. Adv Anim Vet Sci. 2014; 2(5S): 1-4. doi: 10.14737/journal.aavs/2014/2.5s.1.4
26. Warke SR, Ingle VC, Kurkure NV, Tembhurne PA, Prasad M, Chaudhari SP, Barbuddhe SB. Biofilm formation and associated genes in *Listeria monocytogenes*. IJVSBT 2017; 12(3): 7-12. doi: 10.21887/ijvsbt.v12i3.7079
27. EUCAST. European Committee on Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing EUCAST disk diffusion method, 2021, Version 9.0.
28. EUCAST. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, 2022, Version 12.0.
29. CLSI. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, 2017, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA.
30. Magiorakos A-P, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3): 268-281. doi: 10.1111/j.1469-0691.2011.03570.x
31. Sauders BD, Wiedmann M. Ecology of *Listeria* species and *L. monocytogenes* in the natural environment. In: Ryser ET, and Marth EH (eds.), *Listeria, Listeriosis, and Food Safety.* Boca Raton, FL: CRC Press, 2017.
32. Rocourt J, Hof H, Schrettenbrunner A, Malinverni R, Bille J. Méningite purulente aiguë à *Listeria seeligeri* chez un adulte immunocompétent [Acute purulent *Listeria seelingeri* meningitis in an immunocompetent adult]. Schweiz Med Wochenschr. 1986; 116(8): 248-251.
33. Chen J-Q, Regan P, Laksanalamai P, Healey S, Hu Z. Prevalence and methodologies for detection, characterization and subtyping of *Listeria monocytogenes* and *L. ivanovii* in foods and environmental sources. Food Sci Hum Wellness. 2017; 6(3): 97-120. doi: 10.1016/j.fshw.2017.06.002
34. Guillet C, Join-Lambert O, Le Monnier A, Leclercq A, Mechaï F, Mamzer-Bruneel MF, Bielecka MK, Scortti M, Disson O, Berche P, Vazquez-Boland J, Lortholary O, Lecuit M. Human listeriosis caused by *Listeria ivanovii*. Emerg Infect Dis. 2010; 16(1): 136-138. doi: 10.3201/eid1601.091155
35. Beye M, Gouriet F, Michelle C, Casalta JP, Habib G, Raoult D, Fournier PE. Genome analysis of *Listeria ivanovii* strain G770 that caused a deadly aortic prosthesis infection. New Microbes New Infect. 2016; 10: 87-92. doi: 10.1016/j.nmni.2016.01.005
36. Zakrzewski AJ, Chajęcka-Wierzchowska W, Zadernowska A, Podlasz P. Virulence characterization of *Listeria monocytogenes, Listeria innocua*, and *Listeria welshimeri* isolated from fish and shrimp using in vivo early zebrafish larvae models and molecular study. Pathogens 2020; 9(12): 1028. doi: 10.3390/pathogens9121028
37. Lemon KP, Higgins DE, Kolter R. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. J Bacteriol. 2007; 189(12): 4418-4424. doi: 10.1128/JB.01967-06
38. Duan Q, Zhou M, Zhu L, Zhu G. Flagella and bacterial pathogenicity. J Basic Microbiol. 2013; 53(1): 1-8. doi: 10.1002/jobm.201100335
39. Abd El-Malek AM, Ali SFH, Hassanein R, Mohamed AM, Elsayh KI. Occurrence of *Listeria* species in meat, chicken products and human stools in Assiut city, Egypt with PCR use for rapid identification of *Listeria monocytogenes*. Vet World. 2010; 3(8): 353-359.
40. Gray DI, Kroll RG. Polymerase chain reaction amplification of the *flaA* gene for the rapid identification of *Listeria* spp. Lett Appl Microbiol. 1995; 20: 65-68. doi: 10.1111/j.1472-765X.1995.tb00409.x
41. Pereira CS, Santos AJ, Bejerano-Sagie M, Correia PB, Marques JC, Xavier KB. Phosphoenolpyruvate phosphotransferase system regulates detection and processing of the quorum sensing signal autoinducer-2. Mol Microbiol. 2012; 84(1):93-104. doi: 10.1111/j.1365-2958.2012.08010.x
42. Lee S, Çetinkaya F, Soyutemiz G. Investigation on the presence of *Listeria monocytogenes* in some foods produced for exporting. Acta Vet Eurasia 2007; 33(2): 1-11.
43. Aytac SA, Ben U, Cengiz C, Taban BM. Evaluation of *Salmonella* and *Listeria monocytogenes* contamination on leafy green vegetables. J Food Agric Environ. 2010; 8(2): 275-279.

44. Kara R, Acaröz U, Gürler Z, Soylu A, Küçükkurt O. Determination of the presence of *Escherichia coli* O157 and *Listeria monocytogenes* in fresh lettuce samples. EJOSAT. 2019; 16: 870-873. doi: 10.31590/ejosat.573247
45. Szabo EA, Scurrah KJ, Burrows JM. Survey for psychrotrophic bacterial pathogens in minimally processed lettuce. Lett Appl Microbiol. 2000; 30(6): 456-460. doi: 10.1046/j.1472-765x.2000.00747.x
46. Health Canada. Policy on *Listeria monocytogenes* in ready-to-eat foods, 2011. Retrieved from https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/policy-listeria-monocytogenes-ready-eat-foods-2011.html.
47. Campos J, Mourão J, Pestana N, Peixe L, Novais C, Antunes P. Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. Int J Food Microbiol. 2013; 166(3): 464-470. doi: 10.1016/j.ijfoodmicro.2013.08.005
48. Soni DK, Singh M, Singh DV, Dubey SK. Virulence and genotypic characterization of *Listeria monocytogenes* isolated from vegetable and soil samples. BMC Microbiol. 2014; 14: 241. doi: 10.1186/s12866-014-0241-3
49. Goni AM, Tan PL, Esah EM, Chuah LO, Rusul G. Prevalence of *Listeria* species in raw leafy vegetables obtained from wet markets. In: Conference: 33rd MSM Symposium, 14-17 December Kuala Lumpur/Malaysian, 2016, p: 1.
50. Ajayeoba TA, Atanda OO, Obadina AO, Bankole MO, Adelowo OO. The incidence and distribution of *Listeria monocytogenes* in ready-to-eat vegetables in South-Western Nigeria. Food Sci Nutr. 2016; 4(1): 59-66. doi: 10.1002/fsn3.263
51. Leong D, NicAogáin K, Luque-Sastre L, McManamon O, Hunt K, Alvarez-Ordóñez A, Scollard J, Schmalenberger A, Fanning S, O'Byrne C, Jordan K. A 3-year multi-food study of the presence and persistence of *Listeria monocytogenes* in 54 small food businesses in Ireland. Int J Food Microbiol. 2017; 249: 18-26. doi: 10.1016/j.ijfoodmicro.2017.02.015
52. Moravkova M, Verbikova V, Michna V, Babak V, Cahlikova H, Karpiskova R, Kralik P. Detection and quantification of *Listeria monocytogenes* in ready-to-eat vegetables, frozen vegetables and sprouts examined by culture methods and real-time PCR. J Food Nutr Res. 2017; 5(11): 832-837. doi: 10.12691/jfnr-5-11-6
53. Li K, Weidhaas J, Lemonakis L, Khouryieh H, Stone MJ, Jones L, Shen C. Microbiological quality and safety of fresh produce in West Virginia and Kentucky farmers' markets and validation of a post-harvest washing practice with antimicrobials to inactivate *Salmonella* and *Listeria monocytogenes*. Food Control 2017; 79: 101-108. doi: 10.1016/j.foodcont.2017.03.031
54. Chen M, Cheng J, Wu Q, Zhang J, Chen Y, Zeng H, Ye Q, Wu S, Cai S, Wang J, Ding Y. Prevalence, potential virulence, and genetic diversity of *Listeria monocytogenes* isolates from edible mushrooms in Chinese markets. Front Microbiol. 2018; 9: 1711. doi: 10.3389/fmicb.2018.01711
55. Kljujev I, Raicevic V, Jovicic-Petrovic J, Vujovic B, Mirkovic M, Rothballer M. *Listeria monocytogenes*-danger for health safety vegetable production. Microb Pathog. 2018; 120: 23-31. doi: 10.1016/j.micpath.2018.04.034
56. 56. Kyere EO, Qiu GW, Md Zain SN, Palmer J, Wargent JJ, Fletcher GC, Flint S. A comparison of *Listeria monocytogenes* contamination in bagged and un-bagged lettuce in supermarkets. LWT 2020; 134:110022. doi: 10.1016/j.lwt.2020.110022
57. Samad A, Asmat R, Naeem M, Ali H, Mustafa MZ, Abbas F, Raza J, Asmat MT. Isolation and identification of *Listeria monocytogenes* from raw vegetables and meat sold in Quetta, Pakistan. Pak J Zool. 2020; 52(2): 817-820. doi: 10.17582/journal.pjz/20190402110434
58. Abril AG, Carrera M, Böhme K, Barros-Velázquez J, Calo-Mata P, Sánchez-Pérez A, Villa T G. Proteomic characterization of antibiotic resistance in *Listeria* and production of antimicrobial and virulence factors. Int J Mol Sci. 2021; 22(15): 8141. doi: 10.3390/ijms22158141
59. Stonsaovapak S, Boonyaratanakornkit M. Prevalence and antimicrobial resistance of *Listeria* species in food products in Bangkok, Thailand. J Food Saf. 2010; 30: 154-161. doi: 10.1111/j.1745-4565.2009.00197.x
60. Cufaoglu G, Ambarcioglu P, Ayaz ND. Meta-analysis of the prevalence of *Listeria* spp. and antibiotic resistant *L. monocytogenes* isolates from foods in Turkey. LWT. 2021; 144: 111210. doi: 10.1016/j.lwt.2021.111210.

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3860-3871
doi: 10.25083/rbl/28.1/3860.3871

Received for publication, July, 28, 2023
Accepted, August, 14, 2023

Review

Integration of biotechnology and information technology for healthcare innovation

AKINOLA SEGUN[1]

[1] Johanessburg Business School, University of Johannesburg, Johannesburg, South Africa.

Abstract

This review explores the integration of biotechnology and information technology in healthcare innovation. The convergence of these fields has revolutionized diagnostics, therapeutics, and patient management. Biotechnology advancements, such as genomics and molecular diagnostics, enable personalized medicine, while information technology facilitates data management and analysis. The integration also extends healthcare access through telemedicine and remote patient monitoring, enhancing healthcare delivery in underserved areas. Challenges include data security and privacy concerns. Looking ahead, the integration of biotechnology and information technology holds immense potential for further healthcare innovation, transforming patient outcomes and healthcare delivery.

Keywords

-

To cite this article: SEGUN A. Integration of biotechnology and information technology for healthcare innovation. *Rom Biotechnol Lett.* 2023; 28(1): 3860-3871 DOI: 10.25083/rbl/28.1/3860.3871

*Corresponding author: Akinola Segun; email: akinolaa@uj.ac.za

Introduction

The integration of biotechnology and information technology has revolutionized healthcare, leading to significant advancements in diagnosis, treatment, and patient care [1]. This synergistic combination has paved the way for a new era of healthcare innovation, offering tremendous potential for improving health outcomes and transforming the delivery of medical services [2].

Biotechnology involves the application of biological knowledge and techniques to develop products and processes that benefit human health [3]. On the other hand, information technology encompasses the use of computers, software, and data analysis to manage and exchange information effectively. The convergence of these two fields has resulted in ground-breaking solutions that have far-reaching implications for healthcare [4]. The biotechnology application is applied to so many sector for implementations (see Fig. 1) . The key focus is in healthcare and related.

The integration of biotechnology and information technology has enabled the development of innovative medical devices, precision medicine approaches, telehealth services, electronic health records, and sophisticated data analytics tools [5]. These advancements have enhanced medical diagnostics, personalized treatment plans, remote patient monitoring, and streamlined healthcare operations. Moreover, this integration has fostered collaboration among multidisciplinary teams, including healthcare professionals, biologists, engineers, and computer scientists, to address complex healthcare challenges [6].

The significance of this integration for healthcare innovation cannot be overstated. It has facilitated the rapid and accurate diagnosis of diseases, leading to early interventions and improved patient outcomes. By leveraging the power of big data and artificial intelligence, healthcare providers can extract valuable insights from vast amounts of patient information, enabling personalized medicine tailored to individual needs. Furthermore, the integration of biotechnology and information technology has expanded access to healthcare services, particularly in remote and underserved areas, through telemedicine and mobile health applications [7].

The objectives of this review are to examine the current state of integration between biotechnology and information

technology in healthcare, highlight the key innovations and advancements in the field, and assess the impact of this integration on patient care and healthcare systems. We will also explore the challenges and future prospects of this convergence and discuss potential ethical, legal, and social implications.

The scope of this review will encompass various aspects of the integration, including but not limited to:

1. Biotechnology-enabled medical devices and diagnostics
2. Applications of information technology in personalized medicine
3. Telehealth and remote patient monitoring systems
4. Electronic health records and health information exchange
5. Data analytics and artificial intelligence in healthcare
6. Collaborative research and development efforts in biotechnology and information technology
7. Regulatory considerations and ethical implications of the integration.

By comprehensively examining the integration of biotechnology and information technology in healthcare, this review aims to provide insights into the transformative potential of this synergy and its implications for future healthcare innovation.

Biotechnology in Healthcare

Biotechnology plays a pivotal role in revolutionizing healthcare by offering innovative solutions in diagnostics, therapeutics, and personalized medicine [8]. It encompasses a wide range of techniques and technologies that leverage biological systems, living organisms, or their components to develop products and processes that improve human health. In this section, we will explore the multifaceted contributions of biotechnology in healthcare and discuss recent advancements in biotechnological tools and techniques.

Diagnostics

Biotechnology has transformed diagnostic approaches, enabling faster and more accurate identification of diseases. Molecular diagnostics, based on biotechnological tools, have revolutionized disease detection and monitoring [9]. Techniques such as polymerase chain reaction (PCR), gene sequencing, and microarray analysis allow for the identification and characterization of genetic mutations, pathogens, and biomarkers associated with various diseases.

These advancements have paved the way for personalized diagnostics and precision medicine, facilitating targeted therapies and improved patient outcomes [10].

Therapeutics

Biotechnology has revolutionized therapeutic interventions by providing innovative treatment options. Recombinant DNA technology allows for the production of therapeutic proteins, such as insulin, growth factors, and monoclonal antibodies, with high specificity and efficacy [11]. Biopharmaceuticals derived from biotechnology offer enhanced therapeutic potential, addressing complex

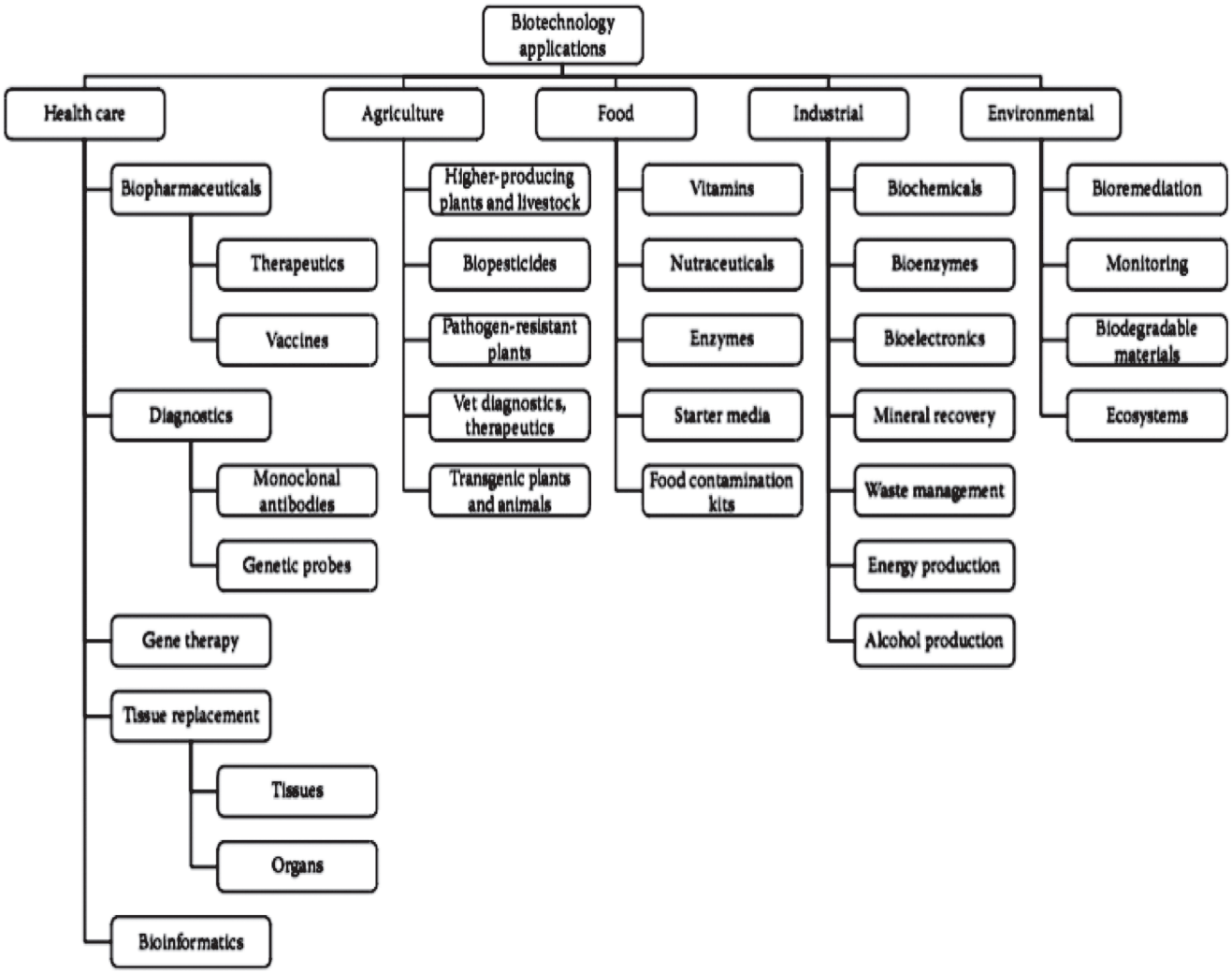


Fig. 1. Application of biotechnology

diseases like cancer, autoimmune disorders, and genetic conditions. Moreover, gene editing techniques, such as CRISPR-Cas9, provide the ability to modify genetic material, opening doors for potential cures for genetic diseases [12].

Personalized Medicine

The integration of biotechnology and information technology has facilitated personalized medicine, tailoring treatment strategies to an individual's specific characteristics. Advances in genomics, proteomics, and metabolomics allow for comprehensive profiling of an individual's genetic makeup, protein expression patterns, and metabolic profiles [13]. This wealth of data enables healthcare providers to develop personalized treatment plans, predict drug responses, and optimize therapeutic outcomes. Pharmacogenomics, for example, utilizes genetic information to determine the most effective and safe medication for an individual, minimizing adverse drug reactions and optimizing treatment efficacy.

Recent Advancements in Biotechnological Tools and Techniques

Next-Generation Sequencing (NGS)

NGS technologies have revolutionized the field of genomics by enabling rapid and cost-effective sequencing of entire genomes [14]. These techniques provide valuable insights into genetic variations, disease-causing mutations, and potential therapeutic targets. NGS has transformed diagnostics, enabling the identification of rare genetic diseases and facilitating early intervention and personalized treatment approaches [15].

Gene Editing

The development of precise gene editing tools, such as CRISPR-Cas9, has revolutionized the field of biotechnology. CRISPR-Cas9 allows for targeted modifications of specific genes, opening up possibilities for correcting genetic defects and developing new therapies [17]. This technique has the potential to treat genetic disorders, enhance disease resistance, and modify cellular functions for therapeutic purposes.

Microarray Technology

Microarray technology enables the simultaneous analysis of thousands of genes or proteins, providing valuable insights into gene expression patterns, protein interactions, and biomarker discovery [18]. Microarrays have facilitated diagnostic advancements, enabling the identification of disease signatures and the development of personalized treatment strategies.

Synthetic Biology

Synthetic biology combines engineering principles with biological components to design and construct new biological systems or modify existing ones [18]. This field has applications in drug development, biofuel production, and the creation of engineered organisms for medical purposes. Synthetic biology offers new avenues for the development of therapeutics, biomaterials, and biotechnological tools [19].

In conclusion, biotechnology plays a pivotal role in healthcare by offering advancements in diagnostics, therapeutics, and personalized medicine. Recent biotechnological tools and techniques, such as next-generation sequencing, gene editing, microarray technology, and synthetic biology, have revolutionized healthcare practices. These advancements hold great promise for improving patient care, developing targeted therapies, and paving the way for precision medicine approaches.

Information Technology in Healthcare

Information technology (IT) has become indispensable in modern healthcare systems, playing a crucial role in patient management, healthcare delivery, and data-driven decision-making. In this section, we will explore the importance of information technology in healthcare systems and discuss key components such as electronic health records (EHRs), health information exchange, and data analytics [20].

Importance of Information Technology in Healthcare Systems:

Information technology has transformed healthcare systems by enhancing efficiency, accuracy, and accessibility of patient information. It enables seamless communication and collaboration among healthcare professionals, improves patient safety, and streamlines administrative processes [21]. Here are some key aspects of the importance of IT in healthcare:

1. Efficient Data Management: IT systems allow healthcare organizations to store, manage, and retrieve vast amounts of patient data efficiently. This includes medical histories, laboratory results, medication records, and imaging studies [22]. Digital storage of patient information eliminates the need for physical records, reduces the risk of data loss, and facilitates quick and secure access to patient data when needed.
2. Improved Patient Safety: IT systems play a critical role in medication management, reducing the risk of errors and adverse drug events. Computerized physician order entry (CPOE) systems and barcode medication administration systems help prevent medication errors by ensuring accurate prescribing, dispensing, and administration of medications [23]. IT systems also enable real-time monitoring of patients' vital signs and automated alerts for abnormal values, enhancing patient safety and timely interventions.
3. Enhanced Communication and Collaboration: IT systems facilitate seamless communication and collaboration among healthcare providers across different locations and disciplines. Electronic communication platforms, such as secure messaging and teleconferencing, enable quick consultations, exchange of medical information, and remote collaborations [24]. This improves care coordination, especially in complex cases requiring multidisciplinary expertise.
4. Accessibility and Telehealth: IT enables remote access to healthcare services through telehealth and telemedicine platforms. Patients can receive virtual consultations, access medical advice, and receive follow-up care from the comfort of their homes. Telehealth has proven particularly beneficial in rural and underserved areas, improving access to healthcare and reducing geographical barriers [25].

Electronic Health Records (EHRs)

EHRs are digital versions of patients' medical records, containing comprehensive information about their health history, diagnoses, treatments, and laboratory results. EHRs offer numerous benefits [26]:

1. Centralized and Comprehensive Information: EHRs consolidate patient information from various sources into a single, accessible platform. This ensures healthcare providers have a comprehensive view of the patient's medical history, enabling informed decision-making and personalized care.
2. Real-time Information: EHRs allow for real-time updates and immediate access to patient data, enabling timely decision-making, reducing duplication of tests, and facilitating better care coordination among healthcare providers.
3. Interoperability: EHR systems aim to be interoperable, enabling seamless exchange of patient information across different healthcare organizations and systems. This promotes care continuity, enables smooth transi-

tions between healthcare settings, and facilitates health information exchange.

Health Information Exchange

Health information exchange (HIE) involves the secure sharing of patient information between different healthcare organizations and systems. Key benefits of HIE include:

1. Coordinated Care: HIE enables healthcare providers to access essential patient information from various sources, such as hospitals, clinics, and laboratories. This promotes care coordination, reduces medical errors, and improves patient outcomes.
2. Emergency Situations: In emergencies, access to a patient's complete medical history through HIE can be life-saving. It provides critical information to healthcare providers who may not have prior knowledge of the patient, facilitating quick and appropriate interventions.
3. Public Health Surveillance: HIE facilitates the collection and analysis of population health data for public health surveillance purposes. It enables the monitoring of disease outbreaks, identification of public health trends, and facilitates targeted interventions and preventive measures.

Data Analytics in Healthcare

Data analytics leverages IT systems and techniques to analyze large volumes of healthcare data and extract valuable insights. It has the potential to transform healthcare in several ways:

1. Predictive Analytics: Data analytics enables the identification of patterns, trends, and risk factors that can predict disease outcomes or complications. This facilitates early interventions, personalized treatment plans, and proactive patient management.
2. Population Health Management: By analyzing population-level data, data analytics helps identify health trends, risk factors, and gaps in healthcare delivery. This supports the development of targeted interventions, preventive strategies, and resource allocation for improved population health outcomes.
3. Quality Improvement: Data analytics enables healthcare organizations to monitor and measure the quality of care provided. It allows for the identification of areas for improvement, benchmarks performance against industry standards, and supports evidence-based decision-making for quality enhancement.

In conclusion, information technology is of paramount importance in healthcare systems and patient management. It enhances efficiency, accuracy, and accessibility of patient information, improves patient safety, and facilitates seamless communication and collaboration among healthcare providers. Key components of IT in healthcare include electronic health records (EHRs), health information exchange, and data analytics, which enable centralized patient information, interoperability, coordinated care, and data-driven decision-making. Leveraging the power of information technology offers immense potential for improving healthcare outcomes, enhancing patient experiences, and transforming healthcare delivery.

Convergence of Biotechnology and Information Technology

The convergence of biotechnology and information technology has led to ground-breaking advancements in healthcare. This convergence brings together the power of biological systems and data-driven technologies, creating synergies that enhance biotechnological processes and revolutionize data management in healthcare [27]. In this section, we will explore the areas of convergence between biotechnology and information technology and discuss how information technology enhances biotechnological processes and data management in healthcare.

Areas of Convergence:

Genomics and Bioinformatics:

The field of genomics, which involves the study of an organism's complete set of DNA (genome), has greatly benefited from information technology [28]. High-throughput DNA sequencing technologies generate vast amounts of genomic data. Bioinformatics, a discipline that combines biology and computer science, utilizes information technology to store, analyze, and interpret this genomic data. It involves developing algorithms, databases, and computational tools to extract valuable insights from genomic data, including identifying disease-causing mutations, predicting drug responses, and understanding the genetic basis of diseases.

Data Integration and Analysis:

The convergence of biotechnology and information technology enables the integration and analysis of diverse biological datasets. By leveraging data integration techniques and sophisticated analytical tools, researchers can combine genomic, proteomic, and metabolomic data to gain a comprehensive understanding of biological systems [29]. This integrated approach helps identify biomarkers, pathways, and therapeutic targets, leading to the development of personalized medicine strategies and targeted therapies.

Computational Modeling and Simulation:

Information technology enables the development of computational models and simulations to predict biological phenomena and optimize biotechnological processes. Computational models can simulate the behavior of biological

systems, such as protein interactions, cellular processes, and drug interactions [30]. These models aid in drug discovery, protein engineering, and optimizing bioprocesses, reducing the need for costly and time-consuming experimental iterations.

Enhancements in Biotechnological Processes:

Accelerating Drug Discovery

Information technology expedites the drug discovery process by facilitating virtual screening, molecular modelling, and structure-based drug design. Advanced computational tools help identify potential drug candidates, predict their efficacy, and optimize their chemical structures [31]. This reduces the time and cost required for preclinical and clinical trials, leading to faster development of novel therapeutics.

Precision Medicine

The convergence of biotechnology and information technology plays a crucial role in precision medicine, tailoring treatments to individual patients based on their unique genetic profiles, environmental factors, and lifestyle choices. Information technology enables the analysis and interpretation of patient data, including genomic information, clinical records, and environmental factors, to develop personalized treatment plans. This improves treatment efficacy, minimizes adverse drug reactions, and optimizes patient outcomes [32].

Bioprocess Optimization

Information technology supports the optimization of bioprocesses involved in biopharmaceutical production. Advanced software systems monitor and control parameters such as temperature, pH, and nutrient supply, ensuring optimal conditions for cell growth and product synthesis. Real-time monitoring, data analytics, and artificial intelligence techniques enable predictive maintenance, process optimization, and yield improvement, reducing costs and enhancing efficiency in bio-manufacturing.

Data Management in Healthcare

Electronic Health Records (EHRs)

Information technology enhances data management in healthcare through the implementation of electronic health records (EHRs). EHRs streamline the collection, storage, and retrieval of patient data, enabling healthcare providers to access comprehensive and up-to-date medical information. EHRs facilitate efficient data sharing among different healthcare settings, improving care coordination and continuity.

Health Information Exchange (HIE)

Health information exchange (HIE) platforms utilize information technology to enable secure sharing of patient data among healthcare organizations [33]. HIE ensures that critical patient information, including medical history, medications, and allergies, is readily accessible to authorized healthcare providers. This improves care transitions, emergency care, and overall patient safety.

Data Analytics and Insights

Information technology enables robust data analytics in healthcare, helping extract valuable insights from large volumes of patient data. Data analytics techniques, such as machine learning and artificial intelligence, can identify patterns, predict disease outcomes, and optimize treatment plans. These insights support evidence-based decision-making, personalized medicine, and population health management.

Privacy and Security

Information technology also plays a crucial role in ensuring the privacy and security of patient data. Robust cybersecurity measures, encryption techniques, access controls, and audit trails are implemented to protect sensitive patient information. Compliance with regulations such as the Health Insurance Portability and Accountability Act (HIPAA) ensures the secure handling of patient data [34].

In conclusion, the convergence of biotechnology and information technology brings significant advancements to healthcare. It enhances biotechnological processes by accelerating drug discovery, enabling precision medicine, and optimizing bioprocesses. Information technology also revolutionizes data management in healthcare through electronic health records, health information exchange, and advanced analytics. The synergy between biotechnology and information technology holds great promise for improving patient care, advancing medical research, and driving innovation in healthcare delivery.

Applications of Integration: Healthcare Innovation through Biotechnology and Information Technology

The integration of biotechnology and information technology has given rise to numerous applications that have transformed healthcare and led to significant innovations [35]. This section will explore specific examples where the convergence of these fields has revolutionized healthcare, including telemedicine, wearable devices, remote patient monitoring, and precision medicine.

Telemedicine

Telemedicine utilizes information technology to provide healthcare services remotely, bridging the gap between patients and healthcare providers. It enables virtual consultations, remote diagnosis, and treatment, bringing healthcare to patients' homes. The integration of biotechnology and information technology in telemedicine has several advantages:

1. Improved Access: Telemedicine overcomes geographical barriers, allowing patients in remote or underserved areas to access healthcare services [36]. It ensures that individuals in rural locations or areas with limited healthcare facilities can receive timely medical advice and consultations.
2. Cost and Time Savings: Telemedicine eliminates the need for travel and reduces wait times, resulting in cost and time savings for patients. It also minimizes the burden on healthcare infrastructure and resources, optimizing their utilization.
3. Remote Monitoring: Telemedicine incorporates biotechnological tools such as wearable devices, sensors, and remote monitoring systems. These devices collect patient data, such as vital signs, glucose levels, or cardiac activity, and transmit it to healthcare providers in real-time [37]. This allows for proactive management of chronic conditions, early detection of complications, and timely interventions.

In a case patient with diabetes can use a wearable device that continuously monitors their blood glucose levels [38]. The device transmits the data to the healthcare provider, who can remotely monitor the patient's condition, provide timely advice on medication adjustments, and offer lifestyle recommendations.

Wearable Devices

Wearable devices, including smartwatches, fitness trackers, and biosensors, have gained popularity for monitoring personal health and wellness. These devices incorporate biotechnological sensors and integrate with information technology platforms, enabling real-time data collection and analysis [39]. The integration of biotechnology and information technology in wearable devices offers several benefits:

1. Health and Activity Monitoring: Wearable devices can track vital signs, physical activity, sleep patterns, and other health-related metrics. These devices provide users with valuable insights into their well-being, promoting healthy habits, and empowering individuals to take charge of their health.
2. Disease Management: Wearable devices can aid in the management of chronic diseases by monitoring symptoms, medication adherence, and activity levels. They enable individuals and healthcare providers to track disease progression, identify triggers, and adjust treatment plans accordingly.
3. Early Detection and Prevention: By continuously monitoring physiological parameters, wearable devices can detect early signs of health issues or abnormalities [40]. Timely alerts and notifications can be sent to healthcare providers or individuals, prompting further evaluation or intervention.

A case wearable fitness tracker can monitor heart rate, sleep patterns, and physical activity levels. By integrating with a smartphone app, it provides users with actionable insights, encouraging exercise, healthy sleep habits, and stress management.

Remote Patient Monitoring

Remote patient monitoring (RPM) utilizes biotechnological sensors and information technology to monitor patients' health outside of traditional healthcare settings [41]. It involves collecting and transmitting data on vital signs, symptoms, or disease-specific metrics to healthcare providers for analysis and intervention. The integration of biotechnology and information technology in RPM offers several advantages:

1. Continuous Monitoring: RPM enables healthcare providers to remotely monitor patients' health parameters on an ongoing basis. This provides a comprehensive view of the patient's condition, facilitates early detection of complications, and enables timely interventions.
2. Improved Outcomes and Patient Engagement: RPM empowers patients to actively participate in their healthcare by monitoring their own health and sharing data with healthcare providers [42]. This fosters patient engagement, adherence to treatment plans, and enhances outcomes.
3. Reduced Hospital Readmissions: By closely monitoring patients post-discharge, RPM can detect early signs of deterioration, allowing for timely interventions and reducing the risk of hospital readmissions.

A case patient recovering from heart surgery can be equipped with a wearable device that continuously monitors their heart rate, blood pressure, and oxygen saturation levels [43]. The data is transmitted to healthcare providers who can remotely monitor the patient's progress, detect any abnormalities, and intervene if necessary.

Precision Medicine

Precision medicine utilizes biotechnological advancements, genomic information, and information technology to tailor medical treatments to individual patients [44]. It

focuses on understanding the genetic, environmental, and lifestyle factors that influence disease development and response to treatments. The integration of biotechnology and information technology in precision medicine offers the following benefits:

1. Personalized Treatment: Precision medicine allows healthcare providers to develop personalized treatment plans based on an individual's genetic profile, biomarker analysis, and clinical data. This facilitates targeted therapies, reduces adverse effects, and optimizes treatment outcomes.
2. Predictive Analytics: Information technology platforms can analyze large-scale genomic and clinical datasets, providing predictive insights into disease susceptibility, treatment response, and disease progression. This helps healthcare providers make informed decisions and tailor interventions accordingly.
3. Research and Drug Development: Biotechnology and information technology are instrumental in precision medicine research and drug development [45]. Computational models, genomic analysis tools, and advanced analytics enable the identification of new drug targets, discovery of biomarkers, and development of targeted therapies.

In oncology, precision medicine utilizes genomic analysis to identify specific mutations or genetic markers that drive a patient's cancer [46]. This information helps healthcare providers select targeted therapies that are more likely to be effective for that individual, improving treatment outcomes.

In conclusion, the integration of biotechnology and information technology has led to transformative applications in healthcare. Telemedicine, wearable devices, remote patient monitoring, and precision medicine are just a few examples where this convergence has revolutionized patient care, disease management, and medical research. These applications enhance access to healthcare, facilitate proactive monitoring, enable personalized treatments, and empower individuals to actively engage in their own health and well-being. The integration of biotechnology and information technology continues to pave the way for innovative solutions that enhance healthcare outcomes and improve quality of life.

Challenges and Ethical Considerations

While the integration of biotechnology and information technology brings remarkable advancements to healthcare, The translation of biotechnology to the commercial development has face several challenges which include (see Fig 1) [47], not limited to it also presents challenges and ethical considerations that need to be addressed. Some of the key challenges and ethical considerations associated [48] with this convergence are:

Privacy and Data Security

The integration of biotechnology and information technology involves the collection, storage, and analysis of vast amounts of sensitive patient data. Maintaining patient privacy and ensuring data security are critical. Healthcare organizations must implement robust cybersecurity measures, encryption techniques, access controls, and data anonymization practices to protect patient information from unauthorized access or breaches. Striking a balance between data sharing for research purposes and preserving patient privacy is a complex challenge that requires careful consideration.

Informed Consent

The integration of biotechnology and information technology often requires the collection and use of personal health data [49]. Obtaining informed consent from patients for the use and sharing of their data is crucial. Patients should be fully informed about how their data will be used, who will have access to it, and the potential benefits and risks involved. Consent mechanisms should be transparent, and patients should have the right to control their data and revoke consent if desired.

Data Accuracy and Reliability

Biotechnological tools generate vast amounts of data, and information technology processes and analyzes this data for medical decision-making [50]. Ensuring the accuracy, reliability, and quality of the data is crucial for sound clinical decisions. Healthcare providers and researchers need to address issues related to data completeness, standardization, and data integrity to minimize errors and biases in data analysis [51].

Health Inequities and Access

The integration of biotechnology and information technology has the potential to exacerbate existing health inequities if access to technology or digital infrastructure is unevenly distributed. Ensuring equitable access to biotechnological tools and information technology platforms is essential to prevent further disparities in healthcare outcomes [52]. Efforts should be made to bridge the digital divide, especially in underserved communities, to ensure that advancements in healthcare benefit all individuals regardless of their socioeconomic status.

Regulatory and Legal Frameworks

The rapid pace of innovation in biotechnology and information technology presents challenges for regulatory and legal frameworks to keep up [53]. Regulations need to be

updated and adapted to address emerging technologies, data privacy concerns, and ethical considerations. It is important to establish clear guidelines and standards for data protection, consent processes, and responsible use of technology to ensure the ethical and responsible integration of biotechnology and information technology in healthcare.

Ethical Use of Data

The integration of biotechnology and information technology generates a wealth of data that holds tremendous potential for medical research and innovation [54]. However, the ethical use of data is paramount. Data should be used in a responsible and transparent manner, with respect for patient privacy and the principles of beneficence and non-maleficence. Safeguards should be in place to prevent the misuse or misinterpretation of data, and data ownership and intellectual property rights should be clearly defined.

Addressing these challenges and ethical considerations requires a collaborative effort from healthcare providers, researchers, policymakers, and regulatory bodies. It is important to establish guidelines, codes of conduct, and best practices that promote the responsible and ethical integration of biotechnology and information technology, ensuring the benefits are maximized while protecting patient rights and privacy. Continuous evaluation and adaptation of ethical frameworks are necessary to keep pace with technological advancements and evolving societal values.

Future Perspectives and Conclusion

The integration of biotechnology and information technology in healthcare is a dynamic field that continues to evolve and hold immense promise for the future. Several future trends and potential advancements are anticipated in this area, including:

Artificial Intelligence (AI) and Machine Learning: AI and machine learning algorithms are expected to play an increasingly important role in analyzing complex biological data, identifying patterns, and making predictions. These technologies have the potential to revolutionize disease diagnosis, drug discovery, and personalized medicine. Internet of Medical Things (IoMT): The IoMT, a network of interconnected medical devices and sensors, will continue to expand, enabling real-time monitoring, remote patient care, and data-driven interventions. This will enhance personalized healthcare, improve patient outcomes, and facilitate proactive disease management. Blockchain Technology: Blockchain technology holds promise in ensuring secure and decentralized storage of healthcare data. It can enhance data privacy, interoperability, and enable secure health information exchange, while giving patients greater control over

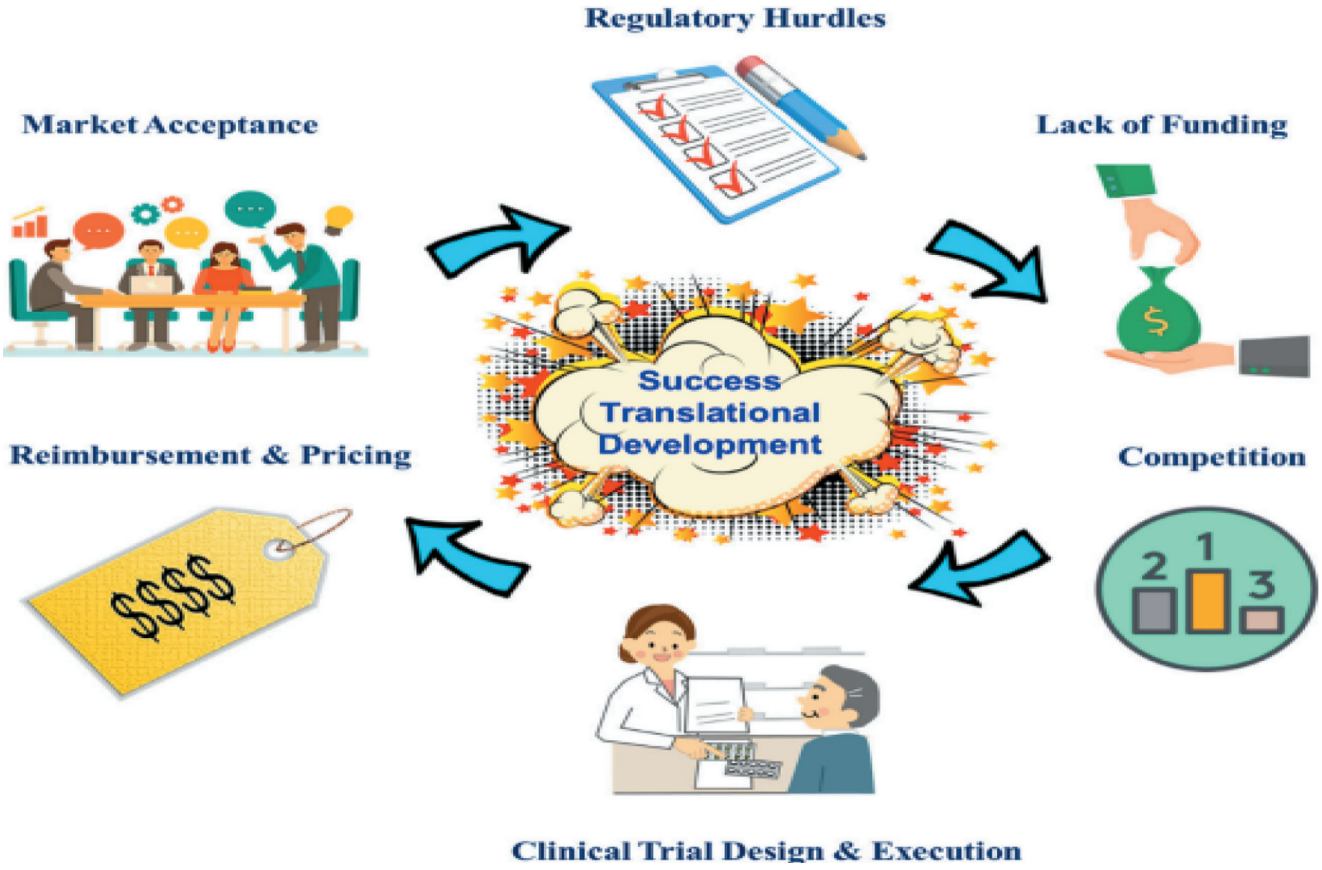


Fig. 2 Translation of biotechnology challenges

their own data.Integration of Omics Data: The integration of genomic, proteomic, metabolomic, and other omics data will advance precision medicine approaches, enabling personalized therapies, predicting disease outcomes, and identifying biomarkers for early detection and intervention. In summary, the integration of biotechnology and information technology has ushered in a new era of healthcare innovation. Throughout this review, we explored the various applications, challenges, and ethical considerations associated with this convergence. We discussed the significant impact this integration has had on healthcare, including the rise of telemedicine, wearable devices, remote patient monitoring, and precision medicine. These advancements have improved access to healthcare, facilitated proactive monitoring, enabled personalized treatments, and empowered individuals to actively engage in their own health and well-being. However, we also recognized the challenges surrounding privacy, data security, consent, and health inequities. It is crucial to address these challenges through robust regulations, ethical frameworks, and responsible data management practices to ensure the responsible integration of biotechnology and information technology in healthcare. looking ahead, the future holds exciting prospects for this integration, with the potential for AI, machine learning, the IoMT, blockchain technology, and omics data integration to further revolutionize healthcare delivery, disease management, and medical research.

In conclusion, the integration of biotechnology and information technology has transformed healthcare, offering unprecedented opportunities for precision medicine, remote patient care, and data-driven decision-making. The synergistic collaboration between these fields has led to groundbreaking innovations, improved patient outcomes, and enhanced healthcare delivery. Embracing this integration, while addressing the associated challenges and ethical considerations, will pave the way for a future where personalized, accessible, and effective healthcare becomes reality for all.

Acknowledgement

I would like to thank Johannesburg business school and University for Johannesburg South Africa

References

4. Ginsburg GS, McCarthy JJ. Personalized medicine: revolutionizing drug discovery and patient care. TRENDS in Biotechnology 2001;19(12):491-496.
5. Seyhan AA., Carini C. Are innovation and new technologies in precision medicine paving a new era in patients centric care? Journal of translational medicine. 2019;17:1-28.
6. Ibañez E, Cifuentes A. Benefits of using algae as natural sources of functional ingredients. Journal of the Science of Food and Agriculture. 2013; 93(4):703-709.
7. Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, van Ijzendoorn MH. Differential susceptibility to the environment: an evolutionary--neurodevelopmental theory. Dev Psychopathol. 2011 Feb;23(1):7-28. doi: 10.1017/S0954579410000611. PMID: 21262036.
8. Kasoju N, Remya NS, Sasi R, Sujesh S, Soman B, Kesavadas C, Muraleedharan CV, Varma PRH, Behari S. Digital health: trends, opportunities and challenges in medical devices, pharma and bio-technology. CSIT. 2023;11(1):11–30. doi: 10.1007/s40012-023-00380-3. Epub 2023 Apr 11. PMCID: PMC10089382.
9. Akinola S, Telukdarie A. Sustainable Digital Transformation in Healthcare: Advancing a Digital Vascular Health Innovation Solution. *Sustainability*. 2023;15(13):10417. doi: 10.3390/su151310417.
10. Källander K, Tibenderana JK, Akpogheneta OJ, Strachan DL, Hill Z, ten Asbroek AH, Conteh L, Kirkwood BR, Meek SR. Mobile health (mHealth) approaches and lessons for increased performance and retention of community health workers in low- and middle-income countries: a review. J Med Internet Res. 2013 Jan 25;15(1):e17. doi: 10.2196/jmir.2130. PMID: 23353680; PMCID: PMC3636306.
11. Dwivedi S, Purohit P, Misra R, Pareek P, Goel A, Khattri S, Pant KK, Misra S, Sharma P. Diseases and Molecular Diagnostics: A Step Closer to Precision Medicine. Indian J Clin Biochem. 2017 Oct;32(4):374-398. doi: 10.1007/s12291-017-0688-8. Epub 2017 Aug 22. PMID: 29062170; PMCID: PMC5634985.
12. Khiyami MA, Almoammar H, Awad YM, Alghuthaymi MA, Abd-Elsalam KA. Plant pathogen nanodiagnostic techniques: forthcoming changes? Biotechnol Biotechnol Equip. 2014 Sep 3;28(5):775-785. doi: 10.1080/13102818.2014.960739. Epub 2014 Oct 22. PMID: 26740775; PMCID: PMC4684063.
13. Seyhan AA, Carini C. Are innovation and new technologies in precision medicine paving a new era in patients centric care? J Transl Med. 2019 Apr 5;17(1):114. doi: 10.1186/s12967-019-1864-9. PMID: 30953518; PMCID: PMC6451233.
14. Sekhon BS. Biopharmaceuticals: an overview. *Thai J. Pharm. Sci* 2010; 34:1-19.

15. Kesik-Brodacka M. Progress in biopharmaceutical development. Biotechnol Appl Biochem. 2018 May;65(3):306-322. doi: 10.1002/bab.1617. Epub 2017 Nov 2. PMID: 28972297; PMCID: PMC6749944.
16. Verma M. Personalized medicine and cancer. J Pers Med. 2012 Jan 30;2(1):1-14. doi: 10.3390/jpm2010001. PMID: 25562699; PMCID: PMC4251363.
17. Xuan J, Yu Y, Qing T, Guo L, Shi L. Next-generation sequencing in the clinic: promises and challenges. Cancer Lett. 2013 Nov 1;340(2):284-95. doi: 10.1016/j.canlet.2012.11.025. Epub 2012 Nov 19. PMID: 23174106; PMCID: PMC5739311.
18. Renkema KY, Stokman MF, Giles RH, Knoers NV. Next-generation sequencing for research and diagnostics in kidney disease. Nat Rev Nephrol. 2014 Aug;10(8):433-44. doi: 10.1038/nrneph.2014.95. Epub 2014 Jun 10. PMID: 24914583.
19. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther. 2020 Jan 3;5(1):1. doi: 10.1038/s41392-019-0089-y. PMID: 32296011; PMCID: PMC6946647.
20. Pontén F, Schwenk JM, Asplund A, Edqvist P-HD. The Human Protein Atlas as a proteomic resource for biomarker discovery. *Journal of internal medicine*. 2011;270(5):428-446.
21. Andrianantoandro E, Subhayu B, David KK, Weiss R. Synthetic biology: new engineering rules for an emerging discipline. *Molecular systems biology*. 2006;2(1):2006-0028.
22. Liu AP, Appel EA, Ashby PD, Baker BM, Franco E, Gu L, Haynes K, Joshi NS, Kloxin AM, Kouwer PHJ, Mittal J, Morsut L, Noireaux V, Parekh S, Schulman R, Tang SKY, Valentine MT, Vega SL, Weber W, Stephanopoulos N, Chaudhuri O. The living interface between synthetic biology and biomaterial design. Nat Mater. 2022 Apr;21(4):390-397. doi: 10.1038/s41563-022-01231-3. Epub 2022 Mar 31. PMID: 35361951; PMCID: PMC10265650.
23. Jha AK, Doolan D, Grandt D, Scott T, Bates DW. The use of health information technology in seven nations. Int J Med Inform. 2008 Dec;77(12):848-54. doi: 10.1016/j.ijmedinf.2008.06.007. Epub 2008 Jul 25. PMID: 18657471.
24. Liu W, Manias E, Gerdtz M. The effects of physical environments in medical wards on medication communication processes affecting patient safety. *Health & Place* . 2014;26:188-198.
25. Dash S, Shakyawar SK, Sharma M, Kaushik S. Big data in healthcare: management, analysis and future prospects. *Journal of big data*. 2019;6(1):1-25.
26. Wang JK, Herzog NS, Kaushal R, Park C, Mochizuki C, Weingarten SR. Prevention of pediatric medication errors by hospital pharmacists and the potential benefit of computerized physician order entry. Pediatrics. 2007 Jan;119(1):e77-85. doi: 10.1542/peds.2006-0034. PMID: 17200262.
27. Rosset C, Rosset A, Ratib O. General consumer communication tools for improved image management and communication in medicine. J Digit Imaging. 2005 Dec;18(4):270-9. doi: 10.1007/s10278-005-6703-2. PMID: 15988626; PMCID: PMC3046724.
28. Shalash A, Spindler M, Cubo E. Global Perspective on Telemedicine for Parkinson's Disease. J Parkinsons Dis. 2021;11(s1):S11-S18. doi: 10.3233/JPD-202411. PMID: 33579872; PMCID: PMC8385495.
29. Burton LC, Anderson GF, Kues IW. Using electronic health records to help coordinate care. Milbank Q. 2004;82(3):457-81, table of contents. doi: 10.1111/j.0887-378X.2004.00318.x. PMID: 15330973; PMCID: PMC2690228.
30. De Guire E, Bartolo L, Brindle R, Devanathan R, Dickey EC, Fessler J, French RH. Data-driven glass/ceramic science research: Insights from the glass and ceramic and data science/informatics communities. *Journal of the American Ceramic Society. 2019;*102 (11):6385-6406.
31. Green ED, Guyer MS; National Human Genome Research Institute. Charting a course for genomic medicine from base pairs to bedside. Nature. 2011 Feb 10;470(7333):204-13. doi: 10.1038/nature09764. PMID: 21307933.
32. Graw S, Chappell K, Washam CL, Gies A, Bird J, Robeson MS 2nd, Byrum SD. Multi-omics data integration considerations and study design for biological systems and disease. Mol Omics. 2021 Apr 19;17(2):170-185. doi: 10.1039/d0mo00041h. PMID: 33347526; PMCID: PMC8058243.
33. Ren LH, Ding YS, Shen YZ, Zhang XF. Multi-agent-based bio-network for systems biology: protein-protein interaction network as an example. Amino Acids. 2008 Oct;35(3):565-72. doi: 10.1007/s00726-008-0081-2. Epub 2008 Apr 19. PMID: 18425405.
34. Willmann S, Lippert J, Schmitt W. From physicochemistry to absorption and distribution: predictive mechanistic modelling and computational tools. Expert Opin Drug Metab Toxicol. 2005 Jun;1(1):159-68. doi: 10.1517/17425255.1.1.159. PMID: 16922658.

35. Vogenberg FR, Isaacson Barash C, Pursel M. Personalized medicine: part 1: evolution and development into theranostics. P T. 2010 Oct;35(10):560-76. PMID: 21037908; PMCID: PMC2957753.
36. Esmaeilzadeh P, Mirzaei T. The Potential of Blockchain Technology for Health Information Exchange: Experimental Study From Patients' Perspectives. J Med Internet Res. 2019 Jun 20;21(6):e14184. doi: 10.2196/14184. PMID: 31223119; PMCID: PMC6610459.
37. Dwyer III SJ, Weaver AC, Hughes KK. Health insurance portability and accountability act. *Security Issues in the Digital Medical Enterprise*. 2004;72(2):9-18.
38. Roco MC, Bainbridge WS. The new world of discovery, invention, and innovation: convergence of knowledge, technology, and society. *Journal of nanoparticle research. 2013;*15:1-17.
39. Krishna VN, Managadi K, Smith M, Wallace E. Telehealth in the Delivery of Home Dialysis Care: Catching up With Technology. Adv Chronic Kidney Dis. 2017 Jan;24(1):12-16. doi: 10.1053/j.ackd.2016.11.014. PMID: 28224937.
40. Yilmaz T, Foster R, Hao Y. Detecting vital signs with wearable wireless sensors. Sensors (Basel). 2010;10(12):10837-62. doi: 10.3390/s101210837. Epub 2010 Dec 2. PMID: 22163501; PMCID: PMC3231103.
41. Lee H, Hong YJ, Baik S, Hyeon T, Kim DH. Enzyme-Based Glucose Sensor: From Invasive to Wearable Device. Adv Healthc Mater. 2018 Apr;7(8):e1701150. doi: 10.1002/adhm.201701150. Epub 2018 Jan 15. PMID: 29334198.
42. Kim J, Campbell AS, de Ávila BE, Wang J. Wearable biosensors for healthcare monitoring. Nat Biotechnol. 2019 Apr;37(4):389-406. doi: 10.1038/s41587-019-0045-y. Epub 2019 Feb 25. PMID: 30804534; PMCID: PMC8183422.
43. Iqbal SMA, Mahgoub I, Du E, Leavitt MA, Asghar W. Advances in healthcare wearable devices. *NPJ Flexible Electronics. 2021;*5(1):9.
44. Atreja A, Francis S, Kurra S, Kabra R. Digital Medicine and Evolution of Remote Patient Monitoring in Cardiac Electrophysiology: A State-of-the-Art Perspective. Curr Treat Options Cardiovasc Med. 2019 Dec 13;21(12):92. doi: 10.1007/s11936-019-0787-3. PMID: 31832887.
45. Sasangohar F, Davis E, Kash BA, Shah SR. Remote Patient Monitoring and Telemedicine in Neonatal and Pediatric Settings: Scoping Literature Review. J Med Internet Res. 2018 Dec 20;20(12):e295. doi: 10.2196/jmir.9403. PMID: 30573451; PMCID: PMC6320401.
46. Patel V, Orchanian-Cheff A, Wu R. Evaluating the Validity and Utility of Wearable Technology for Continuously Monitoring Patients in a Hospital Setting: Systematic Review. JMIR Mhealth Uhealth. 2021 Aug 18;9(8):e17411. doi: 10.2196/17411. PMID: 34406121; PMCID: PMC8411322.
47. Ahmed Z, Mohamed K, Zeeshan S, Dong X. Artificial intelligence with multi-functional machine learning platform development for better healthcare and precision medicine. Database (Oxford). 2020 Jan 1;2020:baaa010. doi: 10.1093/database/baaa010. PMID: 32185396; PMCID: PMC7078068.
48. Blumenthal GM, Mansfield E, Pazdur R. Next-Generation Sequencing in Oncology in the Era of Precision Medicine. JAMA Oncol. 2016 Jan;2(1):13-4. doi: 10.1001/jamaoncol.2015.4503. PMID: 26540172.
49. Gonzalez-Angulo AM, Hennessy BT, Mills GB. Future of personalized medicine in oncology: a systems biology approach. J Clin Oncol. 2010 Jun 1;28(16):2777-83. doi: 10.1200/JCO.2009.27.0777. Epub 2010 Apr 20. PMID: 20406928; PMCID: PMC2881854.
50. Liao C, Xiao S, Wang X. Bench-to-Bedside: Translational Development Landscape of Biotechnology in Healthcare. Health Sciences Review. 2023:100097.
51. Bhuiyan SI. Teaching media convergence and its challenges. *Asia Pacific Media Educator*. 2010;20:115-122.
52. Meingast M, Roosta T, Sastry S. Security and privacy issues with health care information technology. Conf Proc IEEE Eng Med Biol Soc. 2006;2006:5453-8. doi: 10.1109/IEMBS.2006.260060. PMID: 17946702.
53. Kusiak A, Kern JA, Kernstine KH, Tseng BTL. Autonomous decision-making: A data mining approach. *IEEE Transactions on Information technology in Biomedicine*. 2000;4:274-284.
54. Bowman S. Impact of electronic health record systems on information integrity: quality and safety implications. *Perspectives in health information management* 2013;10.
55. Ibrahim H, Liu X, Zariffa N, Morris AD, Denniston AK. Health data poverty: an assailable barrier to equitable digital health care. Lancet Digit Health. 2021 Apr;3(4):e260-e265. doi: 10.1016/S2589-7500-(20)30317-4. Epub 2021 Mar 4. PMID: 33678589.
56. Marchant GE. *The growing gap between emerging technologies and the law*. Springer Netherlands, 2011.
57. Vermesan O, Friess P. *Internet of things applications-from research and innovation to market deployment*. Taylor & Francis, 2014.

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3872-3882
doi: 10.25083/rbl/28.1/3872.3882

Received for publication, July, 28, 2023
Accepted, August, 14, 2023

Original article

Phenotypic virulence and antibiotic resistance features of microbial strains isolated from dental-plaque associated oral lesions

MĂDĂLINA LUPŞA[1,#], MARCELA POPA [1,2,*], MARIA CRAICIU[1,#], ANDREEA DIACONU[1,#], ADELINA-GABRIELA NICULESCU[2,3], CRISTINA TEODORA PREOTEASA[4], MĂDĂLINA BICHERU[4,#], ELENA PREOTEASA[4], IOANA CRISTINA MARINAŞ[2,5]

[1] Faculty of Biology, University of Bucharest, 077206 Bucharest, Romania
[2] Research Institute of the University of Bucharest—ICUB, University of Bucharest, 050657 Bucharest, Romania
[3] Department of Science and Engineering of Oxide Materials and Nanomaterials, Politehnica University of Bucharest, 011061 Bucharest, Romania
[4] Carol Davila University of Medicine and Pharmacy, 8 Eroilor Sanitari Blvd., 050474, Bucharest, Romania
[5] SANIMED INTERNATIONAL IMPEX S.R.L., Romania
[#] These authors have equally contributed to this work as main authors

Abstract

Due to the high complexity of the oral microbial community and its association with diverse oral pathologies, the investigation of microbial resistance and virulence features is essential for developing effective strategies with preventive or therapeutic value. This study focused on identifying a series of soluble virulence factors and the antibiotic resistance profiles of microbial strains isolated from the oral cavity of patients with dental plaque-associated pathologies, using culture-dependent methods. Our study demonstrates that the analyzed bacterial and fungal strains have the ability to grow competitively and induce tissue lesions, mainly mediated by esculinase and proteases (Gram-negative bacilli), hemolysins (*Actinomyces* and aerobic Gram-positive cocci), amylase and DNase (anaerobic Gram-positive cocci), lipase and DNase (yeasts) as well as exhibit resistance to antibiotics currently used in dentistry, such as beta-lactams, tetracyclines and macrolides.

Keywords

virulence factors, antibiotic resistance, oral microbiota.

To cite this article: LUPŞA M. Phenotypic virulence and antibiotic resistance features of microbial strains isolated from dental-plaque associated oral lesions. *Rom Biotechnol Lett.* 2023; 28(1): 3872-3882 DOI: 10.25083/rbl/28.1/3872.3882

*Corresponding author: Marcela Popa. email: marcela.popa@bio.unibuc.ro

Introduction

The oral microbiota represents the second-largest bacterial community after the intestinal microbiota [1] and includes all resident microorganisms of the oral cavity [2-3]. In the oral cavity, microorganisms inhabit the supra- and subgingival tooth surface, tongue, oral mucosa, soft and hard palate, and saliva, with most of the bacteria in saliva being attached to human exfoliated epithelial cells[4]. The human oral microbiome comprises over 2000 taxa of bacteria and fungi co-existing in a complex delicate equilibrium[5-6]. However, under different conditions, such as poor oral hygiene, trauma, broad-spectrum antibiotics, immunosuppression, smoking, and denture wear, an imbalance can occur in the oral microbiota, exposing the organism to various oral and systemic diseases [7-10]. Dental plaque is a non-mineralized biofilm formed by aggregates of resident and/or pathogenic microorganisms and an extracellular matrix of a polymeric nature, structures attached to each other or attached to a solid surface [11-12]. Oral biofilms are the main etiological factor of various oral pathologies, such as dental caries, periodontal diseases, implant-related infections, and oropharyngeal candidiasis [13].

Infection occurs when the virulence, number, and exposure time supersede the local and general host's defense, leading to a pathological reaction in the host's tissues [14]. Virulence is defined as the ability of an organism to infect the host and cause disease. Virulence factors can be secreted extracellularly or associated with the cell envelope, the last category including molecules that allow bacteria to colonize the host at the cellular level [15]. The expression of these virulence factors, and therefore, the microbial pathogenicity level is dependent on the host condition [14, 16]. Hemolysins are virulence factors produced by various bacterial species. These compounds are responsible for cell membrane damage, cell lysis, and cell and tissue destruction to provide nutrients (and iron) to hemolysin-producing bacteria [17]. After spot cultivation on blood agar medium and incubation, hemolysis areas can be observed due to the lysis of red blood cells in the culture medium. Beta (complete) hemolysis is represented by the appearance of a clear, transparent halo around the bacterial colonies [18]. Alpha (partial) hemolysis is represented by the appearance of a pink or green halo around the bacterial colonies. Other extracellular enzymes are caseinase and gelatinase. They are proteases that hydrolyze proteins to peptides and amino acids, destroying the host's tissues and the progression of the infection [18]. Gelatinase is useful in bacterial biofilm formation allowing bacterial cells to aggregate into microcolonies while also being able to destroy the host tissue [19]. Starch is a polysaccharide with a high molecular mass that cannot be transported through the cell membrane to the interior of the bacterial cell, the secretion of extracellular amylases being necessary to achieve its hydrolysis[20]. Esculin (a glucoside) is hydrolyzed to glucose and esculetol. In the presence of iron citrate ($FeC_6H_5O_7$) (Fe^{3+}) in the environment, esculetol released under the action of β-glucosidase (esculinase) leads to the formation of a black precipitate of ferric esculetol, a phenolic compound with Fe^{2+}, whose chemical structure is not fully known [18]. It has been shown that esculetol can fix iron chelators (such as those of the transferrin type), thus providing essential iron ions to bacterial cells to activate genes and express virulence factors. The role of the esculetol is particularly important for extracellular pathogenic bacteria because iron ions are present in small amounts in the extracellular environment, with most of the iron ions circulating in the bound form [18]. To test this virulence factor, the bacterial strains were seeded on esculin containing medium, the presence of a black precipitate around the microbial colonies indicating a positive result. Bacterial deoxyribonucleases (DNases) are enzymes that hydrolyze bacterial nucleic acids producing oligonucleotides used in their syntheses [20-21]. DNases can be involved in several important processes, such as bacterial growth and biofilm maturation, but are also involved in the ability of bacteria to escape the host immune system [20]

Antibiotic resistance is becoming increasingly problematic. It is necessary to know the antibiotic sensitivity or resistance profile of bacterial strains isolated from the oral cavity because bacterial resistance to certain antibiotics can affect the antibiotic treatments recommended in dental conditions. While facing changes in the oral microenvironment, microorganisms can express antibiotic resistance genes, ensuring their survival and genetic persistence, the oral cavity thus becoming a source of antibiotic resistance genes, causing an increase in the number of resistant bacterial infections [22]. Despite the clinical relevance and frequency of dental and oral-maxillofacial infections, there is a lack of recent data on the spectrum of clinical pathogens and associated antimicrobial resistance for those infections [23].

In this regard, this study provides the identification of soluble virulence factors (i.e., hemolysins, lipase, gelatinase, caseinase, lecithinase, esculinase, amylase, and DNase) and the antibiotic resistance profiles of oral cavity-isolated microorganisms, aiming to offer a reliable framework for developing effective preventive and therapeutic strategies against dental pathological conditions.

Materials and Methods

The analyzed bacterial and fungal strains (125) were selected from previously isolates from various oral patholo-

gies, such as dental caries, periodontal diseases, implant-related infections, and oropharyngeal candidiasis, included in the Microbial Collection of the Research Institute of the University of Bucharest.

The identification of soluble virulence factors expressed by bacterial strains was analyzed by cultivation-dependent methods. A bacterial suspension with a density of 0.5 McFarland was obtained from the 24 hour bacterial strains previously cultivated on culture media, which was spotted in a volume of 10 µl on culture media supplemented with a specific substrate for the detection of virulence factors such as hemolysins, lipase, gelatinase, caseinase, lecithinase, esculinase, amylase, and DNase. The inoculated media was incubated for 24 h at 37°C to allow the production and detection of soluble virulence factors, with samples analyzed at 24, 48, and 72 h post-incubation. To evaluate the hemolysin production, the bacterial strains were seeded on blood agar. A positive reaction to the presence of hemolysins is indicated by the presence of a transparent halo around the bacterial colonies, indicating the hemolysis of erythrocytes in the composition of the culture medium. Tween 80 agar was used to evidence the presence of lipase with the formation of an opaque halo around the bacterial colonies, indicating a positive reaction and the absence of the halo a negative reaction [24-25]. Lecithinase was highlighted following cultivation on a culture medium supplemented with egg yolk substrate, the positive reaction being represented by an opaque zone (precipitation) and/or a clear zone around the culture spot. To show the production of proteases, the strains were spotted on solid media with the addition of casein or gelatin, and the presence of a precipitation/clarification zone around the growth area indicated the proteolysis of casein/gelatin (the presence of caseinase/gelatinase) [18, 25]. Esculinase was studied on agar culture medium with esculin by the appearance of a black compound around the bacterial colonies. Agar medium with starch was used to bring out the presence of amylase, and after incubation, Lugol solution was added over the plate with culture medium. The positive result consisted of the appearance of a yellow clarification zone around the bacterial colonies [25]. Bacterial strains were inoculated on agar culture medium with DNA to observe the presence of DNases. A positive response indicating the production of DNase is indicated by the appearance of a yellow halo around the colony on the blue agar.

To establish antibiotic resistance profiles, the inoculum used for seeding was represented by a bacterial suspension made from a pure bacterial culture developed on a solid culture medium (PCA medium), reported on a standard density scale of 0.5 MacFarland or 10^8 CFU/ml. The bacterial inoculum was seeded with a sterile cotton pad after soaking it in bacterial suspension and removing the excess suspension on the tube's inner walls [20]. Antibiotics were placed on the culture medium using a dispenser, after which the plates were incubated at 37°C for 24 hours. Reading the results was carried out by a graduated ruler used to measure the diameters of the inhibition zones around each disc with antibiotic [20].The data obtained from the measurements was reported to standardized tables for the diffusimetric method, recommended by *CLSI* (Clinical and Laboratory Standards Institute). For anaerobic bacteria, the results obtained were reported by the diffusimetric method according to the *EUCAST* (*The European Committee on Antimicrobial Susceptibility Testing*) standard. Based on the results, it was established whether a bacterial strain is sensitive (S), resistant (R), or intermediately sensitive (I) [18].

Results and discussion

Virulence factors

The selected strains were divided into seven microbial groups: aerobic Gram-positive cocci, anaerobic Gram-positive cocci, Gram-positive bacilli, fermentative Gram-negative bacilli, non-fermentative Gram-negative bacilli, anaerobic bacteria, and yeasts. The virulence profiles of different groups are presented in Figure 1.

Regarding the virulence factors for strains belonging to the group of aerobic Gram-positive cocci, hemolysins predominated (Figure 1A). Lipases and lecithinases are included in the category of pore-forming enzymes at the level of the eukaryotic cell membrane leading to the destruction of the lipid content in the membrane structure [18].

Staphylococcus was the predominant genus identified in the gram-positive aerobic cocci-producing hemolysins group. In particular, *S. aureus* is known to exhibit numerous virulence factors such as capsule, coagulase, teichoic acid, polysaccharides, and adhesins; enzymes such as esterases, alpha, beta, gamma, and delta hemolysins, fatty acid modifying enzymes, various proteases, hydrolytic enzymes, catalase, β-lactamase, and various toxins such as leukocidin, enterotoxins, TSST-1 [26-27]. Staphylococcal hemolysins are predominant and the best characterized among the virulence factors expressed by *S. aureus*, being very important in the pathology of staphylococcal infections through their ability to destroy host cells, including cells of the immune system, allowing the spread of bacteria inside the host [27]. The most studied hemolysin from *S. aureus* is α-hemolysin, encoded by the hla gene, which causes lysis of host cells, such as epithelial cells, endothelial cells, erythrocytes, monocytes, and keratinocytes, causing cell membrane damage and their apoptosis [28]. Hemolysin β is a non-pore-forming hemo-

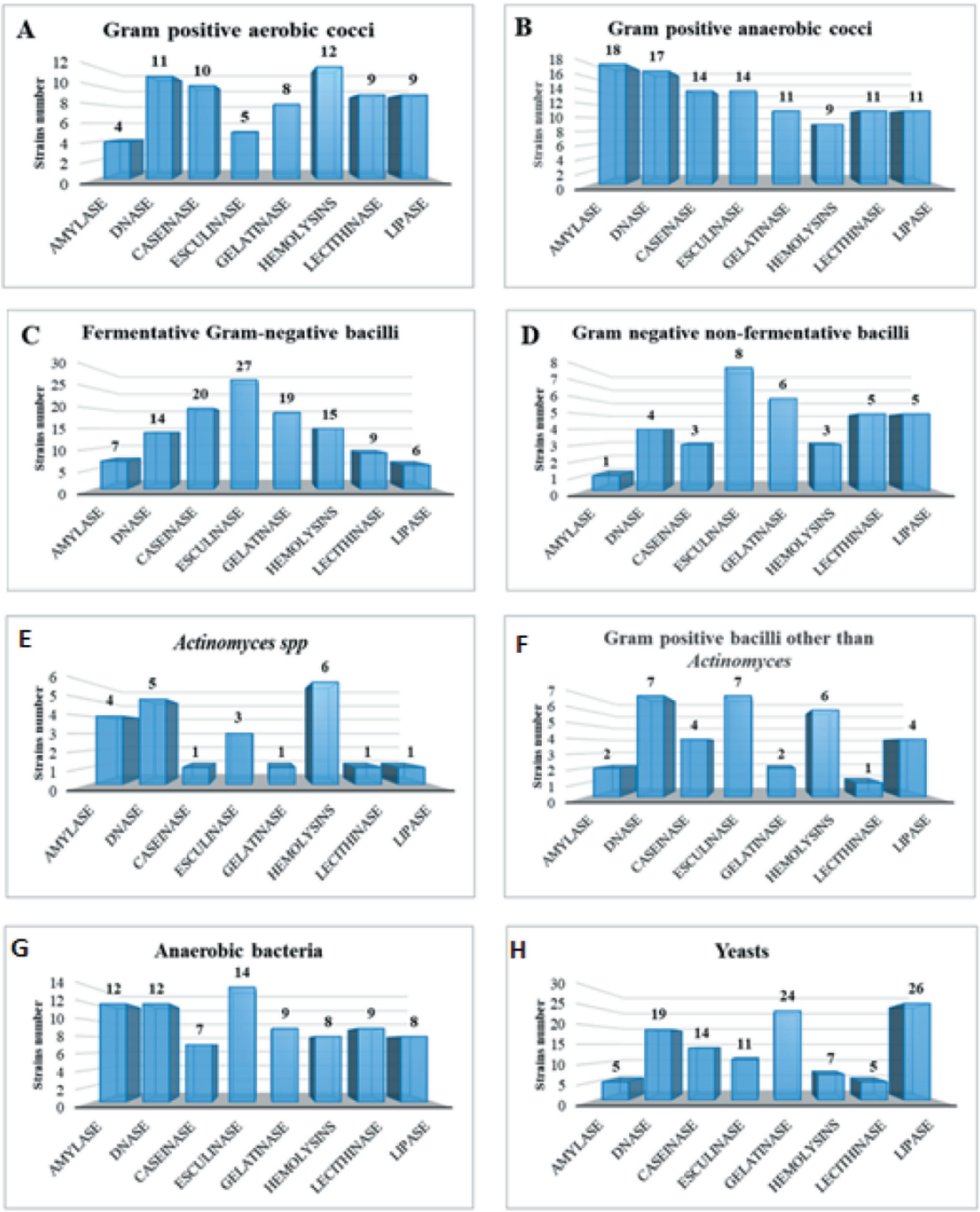


Figure 1. Virulence factors profiles in the analyzed strains. (A) Gram-positive aerobic cocci; (B) Gram positive anaerobic cocci; (C) Fermentative Gram-negative bacilli; (D) Gram-negative non-fermentative bacilli); (E) *Actinymoces* spp; (F) Other Gram-positive bacilli; (G) Anaerobic bacteria; (H) Yeasts.

lysin, a neutral sphingomyelinase secreted by most strains of *S. aureus*, also called warm-cold hemolysin due to the enhanced hemolytic activity observed below 10°C after incubation at 37°C [28]. Gamma hemolysin increases the survival of the bacterial strain of *S. aureus* in human blood, with recent studies showing that strains that present gamma hemolysin are associated with bloodstream infections, including bacteremia and septic arthritis in mouse experiments and endophthalmitis in rabbits [28]. Also, in the study by Kim and Lee, 2015 [29] on *S. aureus* strains isolated from patients with periodontitis, virulence genes for hemolysins were detected, i.e.: hla, hlb, and hld.

Other frequent virulence factors are DNase, caseinase, lecithinase, and lipase, while amylase and esculinase were less frequent in the analyzed strains. Lipase is an important enzyme that has implications for the pathogenesis of some microbial strains, which can form pores in the membranes of eukaryotic cells and alter the lipid content of these cells [18]. *S. aureus* secretes 2 lipases, lipase 1 (SAL1) and lipase 2 (SAL2), encoded by the gehA and gehB genes, respectively. The enzymatic activities of lipases are conferred by the catalytic triad, consisting of serine, aspartate, and histidine [30]. Although they share a similar catalytic mechanism, SAL1 and SAL2 are different, such that the SAL1 lipase functions optimally at pH 6 and is stable under acidic conditions but is inactivated when the pH is above 10. At the same time, the SAL2 lipase functions optimally around pH 8 and is inactive under acidic conditions [30].

In strains of Gram-positive anaerobic cocci, the predominant virulence factors were amylase and DNase (Figure 1B). Esculinase, caseinase, lecithinase, gelatinase, and lipase had medium frequency, while hemosins were identified in fewer strains. The presence of a relatively high percentage of the proteases represented by caseinase and gelatinase could be explained by the presence of the *Streptococcus* genus within this group of bacteria, which is most often associated with the occurrence of dental caries, thus damaging the structure of the dental surface. Starch digestion involves enzymatic degradation, starting at the level of the oral cavity with the formation of maltose and maltodextrins, resulting in a high amount of carbohydrates for the nutrition of oral bacteria [31]. Oral streptococci are the commensal bacteria that colonize the oral cavity and dental plaque biofilm, with some strains exhibiting surface proteins that bind α-amylase, the predominant enzyme in the saliva of many mammalian species [32].Amylase participates in the formation of the salivary film at the level of dental structures, the bacteria developing adhesion mechanisms to the film by binding to different amylase components, which leads to the initiation of bacterial dental plaque formation, which can also facilitate starch metabolism and bacterial development [32]. The amylase binding site is present in the enzyme's glycosylated and non-glycosylated forms [31]. Salivary alpha-amylase exists as monomeric and dimeric forms with calcium and chloride ions, enhancing its enzymatic activity. The ability of α-amylase to bind to microorganisms is a calcium- and enzyme-activity-independent process [31]. Significant evidence supports salivary amylase's role in the production of dental caries. Studies in animals infected with *S. mutans* revealed that a high-starch diet in the absence of sucrose resulted in a lower frequency of caries production; cultivation of the bacterial strain *S. mutans* on the starch substrate in the absence of sucrose produced small amounts of biofilms and glucans on saliva-coated hydroxyapatite discs [33]. *Enterococcus faecalis* can resist antimicrobial substances and survive in a hostile, oligotrophic environment with increased pH that can reach up to 11.5 [34].The association of the bacterial strain *E. faecalis* with the failure of endodontic treatments is due to the ability of this bacteria to invade the dentinal tubules and adhere to the collagen fibers present in the dentin structure [35]. The increased virulence of the *E. faecalis* strain is due to enterococcal surface proteins, aggregating substances, serine proteases, hemolysins, gelatinases, and capsular polysaccharides [35]. Also, *Enterococcus faecalis* has been associated with endodontic infections, and studies by researchers on the virulence factors of this strain have shown that this species expresses factors such as gelatinase and hemolysins. Dahlén et al., 2012 [36], and Komiyama et al., 2016 [37] reported the presence of lipase, hemolysins, and gelatinase, while Khadijeha et al., 2019 [38] reported the presence of extracellular surface proteins and gelatinase.

Esculinase, gelatinase, and caseinase are the dominant virulence factors within the group of fermentative Gram-negative bacilli (Figure 1C). Hemolysins and DNase were present in a moderate percentage, and amylase, lipase, and lecithinase were in a lower number compared to the other virulence factors.

The results obtained for the group of non-fermentative Gram-negative bacilli (Figure 1D) indicated a high frequency for esculinase and gelatinase, moderate for lecithinase, lipase, caseinase, hemolysins, and DNase, and some reduced strains were positive for amylase.

The results obtained regarding the virulence factors for *Actinomyces* strains indicated the predominance of hemolysins (Figure 1E), followed by amylase, DNase, and esculin. Few details are known about the virulence factors produced by *Actinomyces* species but in general; these bacteria are present in polymicrobial communities where the factors produced by *Actinomyces* would contribute to the pathologi-

cal process, these species being involved in the formation of dental plaque; *Actinomyces spp* also interact with other plaque bacteria such as *Fusobacterium*, *Provetella* and *Vieillonella* maintaining the integrity of bacterial plaque [40].

Hemolysins, DNase, and esculinase were the virulence factors for strains of the predominant Gram-positive bacilli genera (Figure 1F). Factors with a moderate frequency are caseinase and lipase, while virulence factors with a low frequency are amylase, gelatinase, and lecithinase. Amylase, especially α-amylase, is an important biological product with wide applications in clinical practice and industry, and for this reason, microorganisms are considered cell suppliers to produce α-amylase, especially *Bacillus subtilis* [41].

The distribution of the virulence factors of the anaerobic bacteria in order of frequency was as follows: esculinase, DNase, amylase, lecithinase, lipase, hemolysins, and caseinase (Figure 1G).

Regarding the virulence factors of yeasts from the identified genera, respectively *Candida* and *Magnusiomyces*, in order of frequency, they are lipase, DNase, caseinase, esculin, hemolysins, amylase, and lecithinase (Figure 1H). The genus *Candida* expresses lipase as a major virulence factor, followed by gelatinase and DNase. The study by Neji et al., 2017 [39] on different strains of *Candida* revealed a high potential of yeast strains to produce caseinase, gelatinase, and hemolysins.

Antibiotics resistance profiles

The oral cavity is colonized by a characteristic and complex microbial community that develops as biofilms on all dental and oral mucosal surfaces [42].The normal microbiota of the oral cavity is associated with various oral pathologies, one of which being periodontitis. Periodontal disease is caused by sessile and planktonic oral microbiota in saliva and dental plaque [43].

According to the protocol followed by clinicians, the treatment of periodontitis involves the mechanical removal of the microbial biofilm that causes inflammation and/or infection. However, in some cases, in addition to the mechanical removal of infected periodontal pockets, the clinical treatment plan for severe forms of periodontitis may involve the adjuvant use of antibiotics [44].

However, the inappropriate use of antibiotics can lead not only to an increase in the frequency of adverse reactions and healthcare costs but also to the risk of selecting antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus (MRSA)*, vancomycin-resistant enterococci (VRE) and multidrug-resistant Gram-negative bacilli [45].

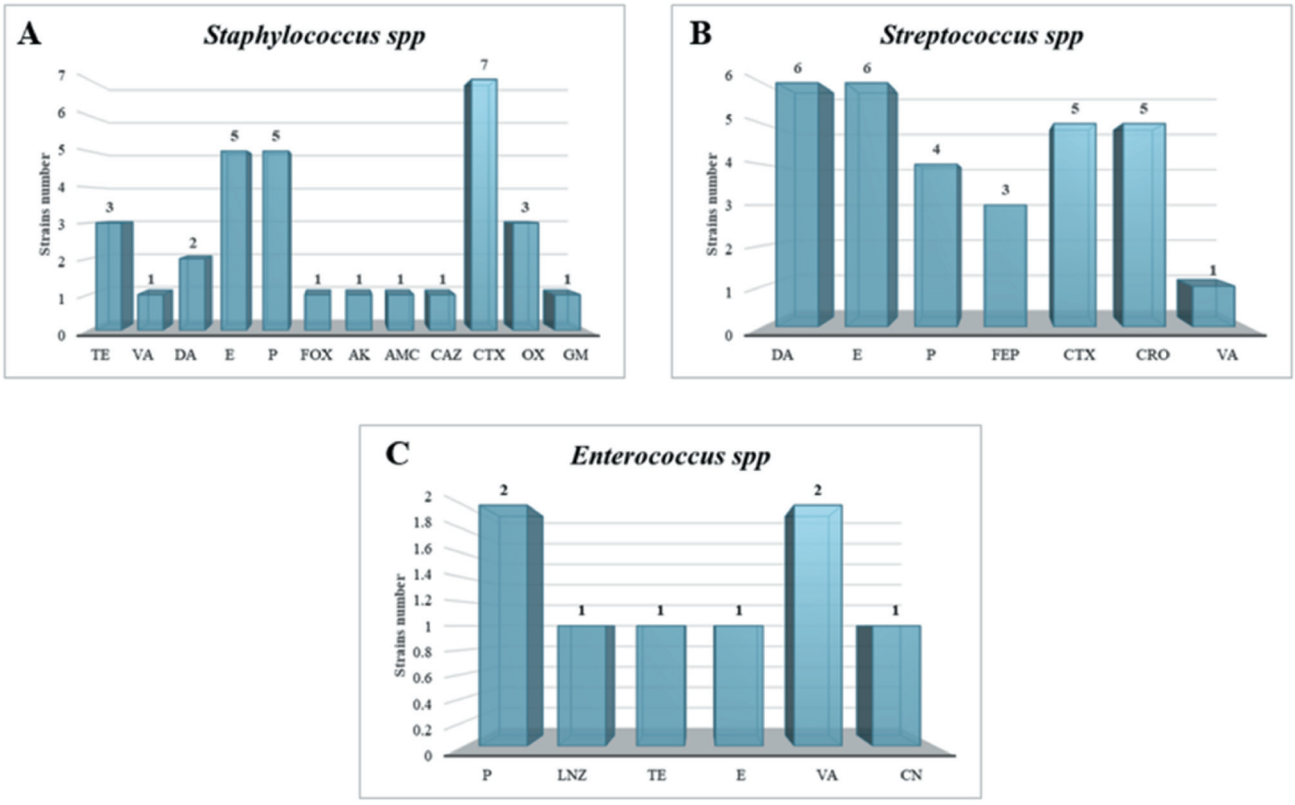


Figure 2. Antibiotic resistance profiles for (A) *Staphylococcus* spp; (B) *Streptococcus* spp; (C) *Enterococcus* spp. Abbreviations: TE - Tetracycline; VA - Vancomycin; DA - Clindamycin; E - Erythromycin; P - Penicillin G; FOX - Cefoxitin; AK - Amikacin; AMC Amoxicillin/ clavulanic acid; CAZ - Ceftazidime; CTX - Cefotaxime; OX - Oxacillin; GM - Gentamicin; FEP - Cefepime; CRO - Ceftriaxone; LNZ - Linezolid; CN - Cefalexin.

Regarding the Gram-positive selected strains, *Staphylococcus spp*. (Figure 2A) were resistant to cefotaxime (n=7), penicillin (n=5) and erythromycin (n=5). Low resistance rates were recorded for antibiotics such as tetracycline, oxacillin, clindamycin, vancomycin, cefoxitin, amikacin, amoxacillin/clavulanic acid, ceftazidime, and gentamicin.

The study carried out by Garbacz et al., 2021 [46] on bacterial strains of staphylococci isolated from the oral cavity revealed that bacterial isolates were resistant to penicillin in a proportion of 62.5%, erythromycin (30.7%), followed by tetracycline (30.2%), cefoxitin/oxacillin (13.5%), clindamycin (15.1%), trimethoprim/sulfamethoxazole (10.4%), fusidic acid (7.8%) and chloramphenicol (4.7%), susceptibility of staphylococci was recorded in the case of vancomycin. Recent studies indicate increased rates of MRSA in the oral cavity [47]. The research carried out by Kim and Lee, 2015 [29] on bacterial strains of *Staphylococcus aureus* isolated from the oral cavity from patients with periodontitis highlighted the fact that most strains were susceptible to vancomycin, chloramphenicol, clindamycin, imipenem, and sulfamethoxazole. The resistance of the analyzed strains was observed in the highest proportion for penicillin and in a lower proportion for oxacillin, erythromycin, tetracycline, and gentamicin. Likewise, the research by Georgiev et al., 2009 [48] on the antibiotic resistance of staphylococci strains isolated from patients with generalized periodontitis revealed sensitivity to gentamicin, concluding that gentamicin is active on aerobic bacteria. In the study carried out by Malinda and Prisinda, 2022 [49] on the susceptibility to antibiotics on bacterial strains isolated from apical abscesses, the sensitivity of *Staphylococcus spp.* strains to penicillin and vancomycin and their resistance to clindamycin were revealed.

In this study, as in other studies, oral staphylococcal strains were resistant to erythromycin and penicillin, to which oxacillin and gentamicin are added in smaller proportions, were identified, with only one MRSA strain being identified.

In the case of the microbial strains belonging to the *Streptococcus* genus, a multi-drug resistance phenotype to antibiotics was observed, with sensitivity being observed for amoxicillin, ofloxacin, linezolid, and tetracycline (Figure 2B). The study carried out by Malinda and Prisinda, 2022 [49] on the susceptibility to antibiotics on bacterial strains isolated from apical abscesses, it revealed the sensi-

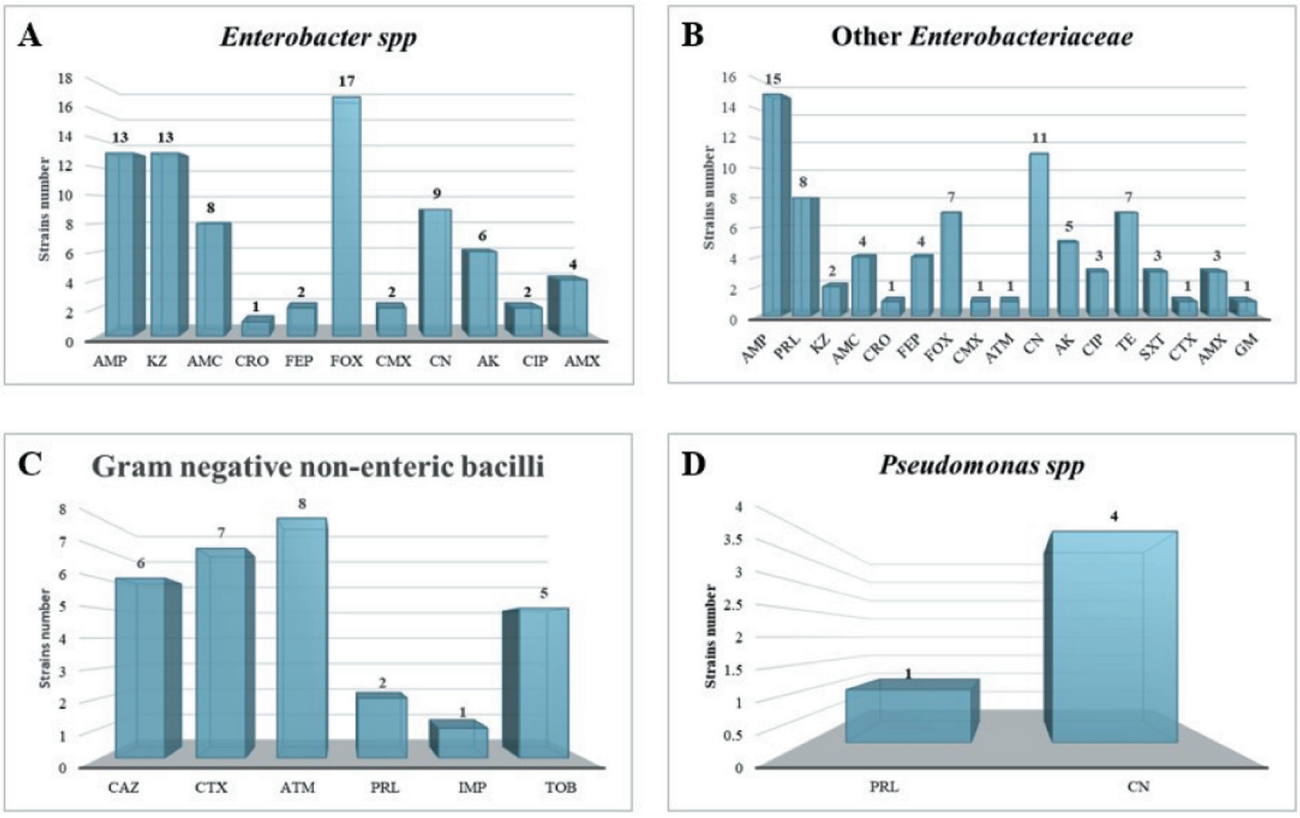


Figure 3. Antibiotics resistance (A) *Enterobacter* spp; (B) Other *Enterobacteriaceae* than *Enterobacter* spp; (C) Gram-negative non-enteric bacilli; (D) *Pseudomonas* spp). Abbreviations: AMP - Ampicillin; KZ - Cefazolin; AMC -Amoxicillin/clavulanic acid; CRO - Ceftriaxone; FEP - Cefepime; FOX - Cefoxitin; CMX - Cefuroxime; CN - Cefalexin; AK - Amikacin; CIP - Ciprofloxacin; AMX - Amoxicillin; PRL - Piperacillin; ATM - Aztreonam; TE - Tetracycline; SXT - Trimethoprim / sulfamethoxazole; CTX - Cefotaxime; GM - Gentamicin; CAZ - Ceftazidime; IMP - Imipenem; TOB - Tobramycin.

tivity of strains belonging to the genus *Streptococcus spp*. to amoxicillin and resistance to clindamycin and vancomycin, similar results being obtained in this work.

Enterococcus strains were predominantly resistant to penicillin, vancomycin, cephalexin, tetracycline, erythromycin, and linezolid and sensitive to ciprofloxacin and chloramphenicol (Figure 2C).Relatively similar results were observed in the study by Komiyama et al., 2016 [37] on *Enterococcus spp.* strains isolated from patients of different ages, which revealed that the strains showed high antibiotic resistance to tetracycline (53.8%), amoxicillin (12.3%), ampicillin (16.0%), erythromycin (43.4%).

The study by Prado et al., 2017 [50] on antibiotic resistance of *E. faecium* and *E. faecalis* strains isolated from root canals revealed that *E. faecalis* was resistant to tetracycline, ciprofloxacin, and azithromycin, while *E. faecium* was sensitive to all antibiotics tested, suggesting that *E. faecium* showed higher susceptibility to antibiotics than *E. faecalis*.

Regarding the selected Gra,-negative isolates, the resistance profile for the strains of the *Enterobacter* genus was analyzed (Figure 3A), and it was observed that the strains of this genus were predominantly resistant to cefoxitin, ampicillin and cefazolin, ciprofloxacin, amoxicillin-clavulanic acid. The study by Jepsen et al., 2022 [51] on bacterial isolates from German periodontitis patients revealed increased resistance of *Enterobacter spp*. strains to ciprofloxacin, and amoxicillin-clavulanic acid, while bacterial isolates from patients with periodontitis from Rio de Janiero were susceptible to ciprofloxacin. In the United States, *Enterobacter* is the second most common genus of carbapenem-resistant *Enterobacteriaceae*, contributing increasingly to the spread of infections with carbapenem-resistant bacteria [52]. Resistance to these antibiotics and the emergence of multidrug resistance have increased interest in these organisms because *Enterobacter cloacae* bacterial strains are nosocomial pathogens capable of producing various infections and septicemia [51].

After analyzing the antibiotic resistance profile of all microbial strains belonging to the *Enterobacteriaceae* family (Figure 3B), it was observed that these strains are resistant in the highest proportion to ampicillin, cephalexin, piperacillin, cefoxitin, and tetracycline. Amoxicillin and moxifloxacin are antibiotics used in the prophylaxis of dental infections after tooth extraction. Diz Dios et al. 2006 [53] showed that the use of amoxicillin and moxiflocin reduced the prevalence and duration of post-extraction bacteremia, the study suggesting that moxiflocin is a promising alternative for the prevention of dental infections. A study carried out on bacterial strains that are part of the *Enterobacteriaceae* family, namely strains of the genera *Enterobacter*, *Klebsiella*, *Serratia*, *Escherichia,* and *Pantoea*, reported similar results regarding resistance to antibiotics such as ampicillin, amoxicillin, amoxicillin-clavulanic acid, cefotoxin, and in the case of imipenem and meropenem, sensitivity was preserved [54]. Also, in this study, it was observed that these strains are beta-lactamase producers, thus suggesting that in the oral cavity of people with endodontic problems could be reservoirs for these enzymatic resistance mechanisms.

Regarding the resistance profile of the microbial strains that are part of the group of non-enteric Gram-negative bacilli, high resistance to ceftazidime, cefotaxime, aztreonam, piperacillin, and imipenem was observed (Figure 3C). Among non-enteric bacilli, *Pseudomonas aeruginosa* strains gain access to the pharynx and oral cavities from external sources or transiently colonize the upper respiratory tract, but their presence in the oral cavity has not been investigated in detail [51]. The strains of the genus *Pseudomonas* isolated in this work were sensitive to almost all tested antibiotics except piperacillin and cephalexin (Figure 3D). In the case of anaerobic bacteria, according to EUCAST, two *Bacteroides urealyticus* bacterial strains were tested and proven susceptible to the two antibiotics (i.e., piperacillin-tazobactam and meropenem).

Conclusions

Regarding the distribution of soluble virulence factors, esculinase and proteases predominated both in the case of non-fermentative and fermentative Gram-negative bacilli, hemolysins were identified in the genus *Actinomyces* and in the case of aerobic Gram-positive cocci, in anaerobic Gram-positive cocci amylase and DNase predominated while in the case of yeast strains, lipase and DNase.

Enterobacteriaceae strains showed high levels of resistance to penicillins (ampicillin) and first generation cephalosporins (cephalexin and cefazolin), non-enteric Gram-negative bacilli to piperacillin, penicillins with inhibitors, carbapenems, aminoglycosides, and quinolones, *Staphylococcus sp*. strains to penicillin and erythromycin, *Enterococcus sp.* strains to vancomycin and penicillin, and *Streptococcus sp.* strains to penicillin, clindamycin, eithromycin. Anaerobic bacterial strains revealed 100% sensitivity to most antibiotics tested.

Acknowledgements

41PFE/30.12.2021/C1.2.PFE-CDI.2021-587/; CNFIS-FDI-2022-0609

References

1. Malan-Müller S, Postolache TT. Editorial: The oral microbiota in mental health. Front Psychiatry. 2022 Oct 7;13:1048179. doi: 10.3389/fpsyt.2022.1048179.

2. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol. 2019 Jan-Apr;23(1):122-128. doi: 10.4103/jomfp.JOMFP_304_18.
3. Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S, Franchi M. Defining the oral microbiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiol. 2020 May 18;20(1):120. doi: 10.1186/s12866-020-01801-y.
4. Chattopadhyay I, Verma M, Panda M. Role of Oral Microbiome Signatures in Diagnosis and Prognosis of Oral Cancer. Technol Cancer Res Treat. 2019 Jan 1;18:1533033819867354. doi: 10.1177/1533033819867354.
5. Salehi B, Kregiel D, Mahady G, Sharifi-Rad J, Martins N, Rodrigues CF. Management of *Streptococcus mutans-Candida spp.* Oral Biofilms' Infections: Paving the Way for Effective Clinical Interventions. J Clin Med. 2020 Feb 14;9(2):517. doi: 10.3390/jcm9020517.
6. Willis JR, Gabaldón T. The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems. Microorganisms. 2020 Feb 23;8(2):308. doi: 10.3390/microorganisms8020308.
7. Zhang Y, Wang X, Li H, Ni C, Du Z, Yan F. Human oral microbiota and its modulation for oral health. Biomed Pharmacother. 2018 Mar;99:883-893. doi: 10.101
8. Coll PP, Lindsay A, Meng J, Gopalakrishna A, Raghavendra S, Bysani P, O'Brien D. The Prevention of Infections in Older Adults: Oral Health. J Am Geriatr Soc. 2020 Feb;68(2):411-416. doi: 10.1111/jgs.16154.
9. Krüger W, Vielreicher S, Kapitan M, Jacobsen ID, Niemiec MJ. Fungal-Bacterial Interactions in Health and Disease. Pathogens. 2019 May 21;8(2):70. doi: 10.3390/pathogens8020070.
10. Sharma VK, Tailor M, Chaudhary VK, Rawat DS, Verma PC, Singh BK. Fungal infections in Otorhinolaryngology: A Descriptive Study. International Multispeciality Journal of Health 2017, 3, 117-124.
11. Jiao Y, Tay FR, Niu LN, Chen JH. Advancing antimicrobial strategies for managing oral biofilm infections. Int J Oral Sci. 2019 Oct 1;11(3):28. doi: 10.1038/s41368-019-0062-1.
12. Drotleff B, Roth SR, Henkel K, Calderón C, Schlotterbeck J, Neukamm MA, Lämmerhofer M. Lipidomic profiling of non-mineralized dental plaque and biofilm by untargeted UHPLC-QTOF-MS/MS and SWATH acquisition. Anal Bioanal Chem. 2020 Apr;412(10):2303-2314. doi: 10.1007/s00216-019-02364-2.
13. Bertolini M, Costa RC, Barão VAR, Villar CC, Retamal-Valdes B, Feres M, Silva Souza JG. Oral Microorganisms and Biofilms: New Insights to Defeat the Main Etiologic Factor of Oral Diseases. Microorganisms. 2022; 10(12):2413. https://doi.org/10.3390/microorganisms10122413.
14. Dahlen G, Basic A, Bylund J. Importance of Virulence Factors for the Persistence of Oral Bacteria in the Inflamed Gingival Crevice and in the Pathogenesis of Periodontal Disease. J Clin Med. 2019 Aug 29;8(9):1339. doi: 10.3390/jcm8091339.
15. Sharma AK, Dhasmana N, Dubey N, Kumar N, Gangwal A, Gupta M, Singh Y. Bacterial Virulence Factors: Secreted for Survival. Indian J Microbiol. 2017 Mar;57(1):1-10. doi: 10.1007/s12088-016-0625-1.
16. Casadevall A, Pirofski LA. Virulence factors and their mechanisms of action: the view from a damage-response framework. J Water Health. 2009;7 Suppl 1:S2-S18. doi: 10.2166/wh.2009.036. PMID: 19717929.
17. Mogrovejo DC, Perini L, Gostinčar C, Sepčić K, Turk M, Ambrožič-Avguštin J, Brill FHH, Gunde-Cimerman N. Prevalence of Antimicrobial Resistance and Hemolytic Phenotypes in Culturable Arctic Bacteria. Front Microbiol. 2020 Apr 3;11:570. doi: 10.3389/fmicb.2020.00570.
18. Chifiriuc C. M., Mihăescu G., Lazăr V., 2011. Microbiologie şi virologie medicală. Editura Universităţii din Bucureşti.
19. Hashem Q. A Conservative Management Approach for Unusual Presentation of Oral Actinomycosis. Case Rep Dent. 2021 May 18;2021:5570758. doi: 10.1155/2021/5570758
20. Lazar V, Herlea V, Cernat R, Bulai D, Balotescu M, Moraru M. Microbiologie generala manual de lucrări practice. Editura Universităţii Bucuresti, 2004.
21. Haas B, Bonifait L, Vaillancourt K, Charette SJ, Gottschalk M, Grenier D. Characterization of DNase activity and gene in *Streptococcus suis* and evidence for a role as virulence factor. BMC Res Notes. 2014 Jul 4;7:424. doi: 10.1186/1756-0500-7-424.
22. Almeida VSM, Azevedo J, Leal HF, Queiroz ATL, da Silva Filho HP, Reis JN. Bacterial diversity and prevalence of antibiotic resistance genes in the oral microbiome. PLoS One. 2020 Sep 29;15(9):e0239664. doi: 10.1371/journal.pone.0239664.
23. Meinen A, Reuss A, Willrich N, Feig M, Noll I, Eckmanns T, Al-Nawas B, Markwart R. Antimicrobial Resistance and the Spectrum of Pathogens in Dental and Oral-Maxillofacial Infections in Hospitals and Dental Practices in Germany. Front Microbiol. 2021 Jun 2;12:676108. doi: 10.3389/fmicb.2021.676108.

24. Mitache MM, Curutiu C, Gheorghe I, Lazar V, Bucur M, Bleotu C, Cochior D, Rusu E, Chifiriuc CM. Phenotypic, Biochemical and Genotypic Charcaterisation of Some Factors Involved in the Virulence and Survival of Bacteria Isolated from Food and Food Processing Surfaces. Rev. Chim. 2018 Jan;69(1):116-120.
25. Preda M, Mihai MM, Popa LI, Dițu LM, Holban AM, Manolescu LSC, Popa GL, Muntean AA, Gheorghe I, Chifiriuc CM, Popa MI. Phenotypic and genotypic virulence features of staphylococcal strains isolated from difficult-to-treat skin and soft tissue infections. PLoS One, 16(2):e0246478.
26. Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. Expression of virulence factors by *Staphylococcus aureus* grown in serum. Appl Environ Microbiol. 2011 Nov;77(22):8097-105. doi: 10.1128/AEM.05316-11.
27. Divyakolu S, Chikkala R, Ratnakar KS, Sritharan V. Hemolysins of *Staphylococcus aureus*—an update on their biology, role in pathogenesis and as targets for anti-virulence therapy. Advances in Infectious Diseases, 2019, 9(2), 80-104.
28. Ahmad-Mansour N, Loubet P, Pouget C, Dunyach-Remy C, Sotto A, Lavigne JP, Molle V. *Staphylococcus aureus* Toxins: An Update on Their Pathogenic Properties and Potential Treatments. Toxins (Basel). 2021 Sep 23;13(10):677. doi: 10.3390/toxins13100677.
29. Kim GY, Lee CH. Antimicrobial susceptibility and pathogenic genes of *Staphylococcus aureus* isolated from the oral cavity of patients with periodontitis. J Periodontal Implant Sci. 2015 Dec;45(6):223-8. doi: 10.5051/jpis.2015.45.6.223
30. Tam K, Torres VJ. *Staphylococcus aureus* secreted toxins and extracellular enzymes. Microbiology Spectrum, 2019, 7(2):10.1128/microbiolspec.GPP3-0039-2018. doi: 10.1128/microbiolspec.
31. Lahiri D, Nag M, Banerjee R, Mukherjee D, Garai S, Sarkar T, Dey A, Sheikh HI, Pathak SK, Edinur HA, Pati S, Ray RR. Amylases: Biofilm Inducer or Biofilm Inhibitor? Front Cell Infect Microbiol. 2021 Apr 27;11:660048. doi: 10.3389/fcimb.2021.660048.
32. Haase EM, Kou Y, Sabharwal A, Liao YC, Lan T, Lindqvist C, Scannapieco FA. Comparative genomics and evolution of the amylase-binding proteins of oral streptococci. BMC Microbiol. 2017 Apr 20;17(1):94. doi: 10.1186/s12866-017-1005-7.
33. Culp DJ, Robinson B, Cash MN. Murine Salivary Amylase Protects Against Streptococcus mutans-Induced Caries. Front Physiol. 2021 Jul 2;12:699104. doi: 10.3389/fphys.2021.699104.
34. Alghamdi F, Shakir M. The Influence of Enterococcus faecalis as a Dental Root Canal Pathogen on Endodontic Treatment: A Systematic Review. Cureus. 2020 Mar 13;12(3):e7257. doi: 10.7759/cureus.7257.
35. Colaco AS. Extreme resistance of Enterococcus faecalis and its role in endodontic treatment failure. Progress in Medical Sciences, 2018, 2(1), 9-13.
36. Dahlén G, Blomqvist S, Almståhl A, Carlén A. Virulence factors and antibiotic susceptibility in enterococci isolated from oral mucosal and deep infections. *J Oral Microbiol.* 2012;4. doi: 10.3402/jom.v4i0.10855.
37. Komiyama EY, Lepesqueur LS, Yassuda CG, Samaranayake LP, Parahitiyawa NB, Balducci I, Koga-Ito CY. *Enterococcus* Species in the Oral Cavity: Prevalence, Virulence Factors and Antimicrobial Susceptibility. PLoS One. 2016 Sep 15;11(9):e0163001. doi: 10.1371/journal.pone.0163001.
38. Khadijeha N, Khudaverdic G, Pouryab G, Asghard T, Mohammad Ahangarzadehe R, Suhad Saadf M, Mohammadg A, Hossein Samadi K. Oral cavity infection by *Enterococcus faecalis* virulence factors and pathogenesis. Reviews in Medical Microbiology, January 18, 2019 - Volume Publish Ahead of Print.
39. Neji S, Hadrich I, Trabelsi H, Abbes S, Cheikhrouhou F, Sellami H, Makni F, Ayadi A. Virulence factors, antifungal susceptibility and molecular mechanisms of azole resistance among *Candida parapsilosis* complex isolates recovered from clinical specimens. J Biomed Sci. 2017 Sep 4;24(1):67. doi: 10.1186/s12929-017-0376-2.
40. Könönen E, Wade WG. Actinomyces and related organisms in human infections. Clinical Microbiology Reviews, 2015, 28(2):419-42.
41. Yan S. Wu G. Bottleneck in secretion of α-amylase in *Bacillus subtilis*. Microbial Cell Factories, 2017, 16, 124. https://doi.org/10.1186/s12934-017-0738-1.
42. Kozak M, Pawlik A. The Role of the Oral Microbiome in the Development of Diseases. Int J Mol Sci. 2023 Mar 9;24(6):5231. doi: 10.3390/ijms24065231.
43. Morrison AG, Sarkar S, Umar S, Lee STM, Thomas SM. The contribution of the human oral microbiome to oral disease: a review. Microorganisms, 2023, 11(2):318.
44. Abe FC, Kodaira K, Motta CCB, Barberato-Filho S, Silva MT, Guimarães CC, Martins CC, Lopes LC. Antimicrobial resistance of microorganisms present in periodontal diseases: A systematic review and meta-analysis. Front Microbiol. 2022 Oct 3;13:961986. doi: 10.3389/fmicb.2022.961986.
45. Tacconelli E, Cataldo MA, Paul M, Leibovici L, Kluytmans J, Schröder W, Foschi F, De Angelis G, De Waure

C, Cadeddu C, Mutters NT, Gastmeier P, Cookson B. STROBE-AMS: recommendations to optimise reporting of epidemiological studies on antimicrobial resistance and informing improvement in antimicrobial stewardship. BMJ Open, 2016, 6(2):e010134.

46. Garbacz K, Jarzembowski T, Kwapisz E, Daca A, Witkowski J. Do the oral Staphylococcus aureus strains from denture wearers have a greater pathogenicity potential? J Oral Microbiol. 2018 Oct 23;11(1):1536193. doi: 10.1080/20002297.2018.1536193.
47. Donkor ES, Kotey FC. Methicillin-Resistant Staphylococcus aureus in the Oral Cavity: Implications for Antibiotic Prophylaxis and Surveillance. Infect Dis (Auckl). 2020 Dec 14;13:1178633720976581. doi: 10.1177/1178633720976581.
48. Georgiev T, Nogalchev K, Georgiev K. Rational antibiotic therapy in the complex treatment of patients with generalized periodontitis. Journal of IMAB Annual Proceeding, 2009, 15 (2), 10-13.
49. Malinda Y, Prisinda D. The antibiotics sensitivity test on *Staphylococcus* and *Streptococcus* from chronic apical abscess. Odonto: Dental Journal, 2022, 9(1). DOI: http://dx.doi.org/10.30659/odj.9.1.130-137.
50. Prado ACC, De Barros PP, Santos JDD, Oliveira LDD, Carvalho CAT, Valera MC, Jorge AOC, Junqueira JC. *Enterococcus faecium* and *Enterococcus faecalis* in endodontic infections: antibiotic resistance profile and susceptibility to photodynamic therapy. Lasers in Dental Scien*ce*, 2017 1, 91–99.
51. Jepsen K, Falk W, Brune F, Cosgarea R, Fimmers R, Bekeredjian-Ding I, Jepsen S. Prevalence and Antibiotic Susceptibility Trends of Selected *Enterobacteriaceae*, *Enterococci*, and *Candida albicans* in the Subgingival Microbiota of German Periodontitis Patients: A Retrospective Surveillance Study. Antibiotics (Basel). 2022 Mar 14;11(3):385. doi: 10.3390/antibiotics11030385.
52. Wilson BM, El Chakhtoura NG, Patel S, Saade E, Donskey CJ, Bonomo RA, Perez F. Carbapenem-Resistant *Enterobacter cloacae* in Patients from the US Veterans Health Administration, 2006-2015. Emerg Infect Dis. 2017 May;23(5):878-880. doi: 10.3201/eid2305.162034.
53. Diz Dios P, Tomás Carmona I, Limeres Posse J, Medina Henríquez J, Fernández Feijoo J, Alvarez Fernández M. Comparative efficacies of amoxicillin, clindamycin, and moxifloxacin in prevention of bacteremia following dental extractions. Antimicrob Agents Chemother. 2006 Sep;50(9):2996-3002. doi: 10.1128/AAC.01550-05.
54. Gaetti-Jardim EC, Marqueti AC, Faverani LP, Gaetti-Jardim E Jr. Antimicrobial resistance of aerobes and facultative anaerobes isolated from the oral cavity. J Appl Oral Sci. 2010 Dec;18(6):551-9. doi: 10.1590/s1678-77572010000600004.

ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2023; 28(1): 3883-3892
doi: 10.25083/rbl/28.1/3883.3892

Received for publication, August, 07, 2023
Accepted, August, 18, 2023

Review

Various facets of low-grade appendiceal mucinous neoplasms (LAMNs)

MARIAN CONSTANTIN[1,2,*]

[1] Institute of Biology of Romanian Academy, 060031, Bucharest, Romania
[2] Fellow of The Research Institute of the University of Bucharest, ICUB, 050095 Bucharest, Romania

Abstract

Low-grade appendiceal mucinous neoplasms are rare tumors of the appendix that affect women and men equally from the fifth decade of life. They are characterized by the replacement of normal appendiceal mucosal tissue with villous proliferations of mucinous epithelium. The tumor cells secrete mucin, which accumulates in intracytoplasmic vacuoles. Tumor growth occurs by pushing mechanisms without invasion, invasion defining adenocarcinomas. In the early stages, these tumors have low risk of recurrence and are not life-threatening, appendectomy being sufficient for cure. Sometimes, the accumulation of mucin produces ruptures of the appendiceal wall, which may seed tumor content outside the appendix, complicating diagnosis and prognosis, presenting a high risk of recurrence and, in the case of pseudomyxoma peritonei, becoming disabling and life-threatening. For these, treatment becomes more complex, with decreased survival rate.

Keywords

low-grade appendiceal mucinous neoplasms (LAMNs); appendix; pseudomyxoma peritonei; signaling pathways; metastasis

To cite this article: CONSTANTIN M. Various facets of low-grade appendiceal mucinous neoplasms (LAMNs). *Rom Biotechnol Lett.* 2023; 28(1): 3883-3892 DOI: 10.25083/rbl/28.1/3883.3892

*Corresponding author: Marian Constantin. email: cvgmarian@gmail.com

Introduction

Low-grade appendiceal mucinous neoplasms (LAMNs) are rare epithelial neoplasms of the cecal appendix, accounting for less than 1% of gastrointestinal tract neoplasms and approximately 0.3% of appendiceal neoplasms [1, 2, 3]. These are heterogeneous diseases of unknown etiology, occurring in the sixth or seventh decade of life and have a female to male ratio of ~1. One study, published by Akay et al. [4] indicates a lower age for males than females and the overall average. Thus, they present cases of low-grade appendiceal mucinous neoplasms with mean ages of 48.6 years (55.4 years for women and 41.4 years for men), indicating that this type of tumor may occur earlier than initially thought. Low-grade appendiceal mucinous neoplasms (LAMNs) are characterized by well-differentiated tumors with proliferation of the appendiceal mucosal epithelium, extracellular mucinous secretion and pushing tumor margins. Being frequently asymptomatic and without causing discomfort, low-grade appendiceal mucinous neoplasms are difficult to diagnose before appendectomy for appendicitis [5]. However, in some cases, there may be abdominal pain and distention, or a palpable mass may be identified on abdominal or pelvic examination, but these are not mandatory. In almost half of the cases, calcifications of the appendiceal wall may occur. Low-grade appendiceal mucinous neoplasms are considered to have variable malignant potential and are generally perceived as semi-malignant tumors. They lack classical invasiveness, often being confined to the appendiceal wall, sometimes perforating it, seeding the peritoneal cavity with neoplastic mucinous cells, acquiring malignant potential and causing pseudomyxoma peritonei [6, 7, 8].

Anatomopathological features

Low-grade appendiceal mucinous neoplasms are a unique subtype of appendiceal tumors, which, under the microscope, take several forms, all of which have low atypia, resemble low-grade colonic dysplasia, and often lack lymphoid tissue. The typical form of low-grade appendiceal mucinous neoplasm is characterized by the replacement of normal mucosal tissue with villous filiform mucinous epithelial proliferations (Figure 1). The tumor cells begin to secrete excess mucin, which becomes accumulated in the cytoplasm as vacuoles, the increased volume and number of vacuoles compressing the nuclei. Other forms of low-grade appendiceal mucinous neoplasms present mucosa with a wavy or scalloped appearance, columnar epithelial cells having nuclear pseudo-stratification and growing on fibrotic submucosal tissue, whereas other forms present the mucinous epithelium as a flattened or attenuated monolayer [6, 7, 9, 10, 11]. Frequently, the appendix wall may have varying degrees of hyalinization, calcification and fibrosis, with epithelial proliferation increasing within this tissue, partially or completely invading the appendix wall structures. Invasion of the appendiceal wall is destructive, confluent, cribriform and with desmoplasia, indicating infiltrative growth and leading to the diagnosis of appendiceal mucinous adenocarcinoma. Fibrosis of the appendix wall structures makes it difficult to identify its layers and, consequently, to assess the

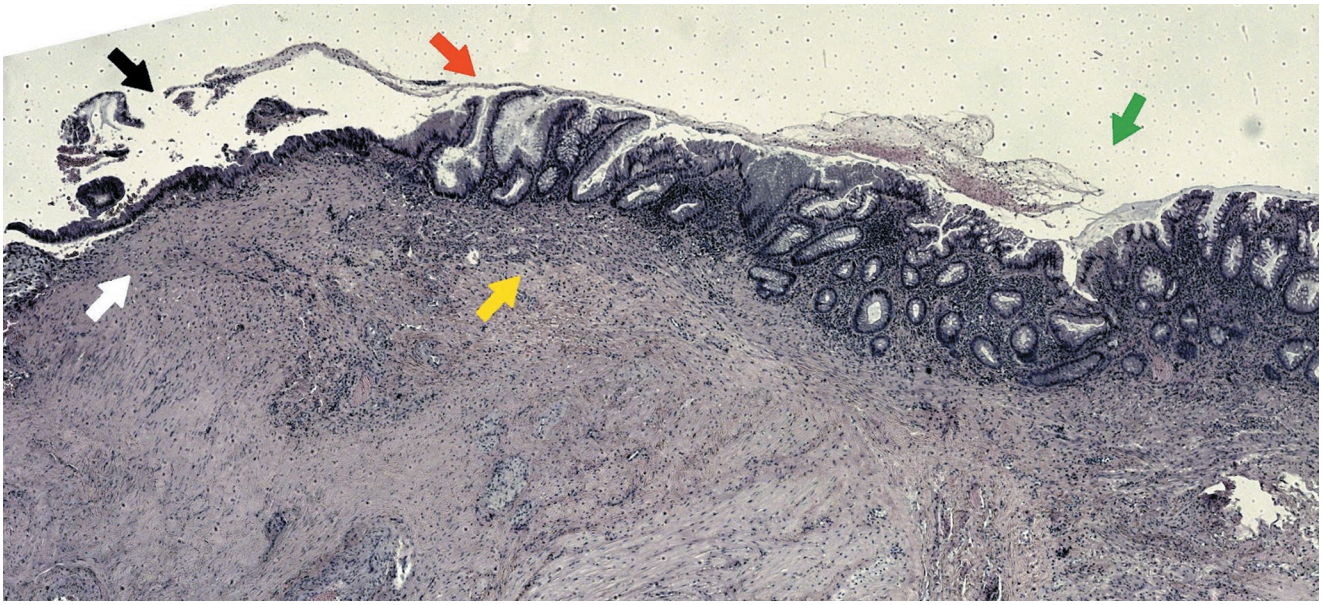


Figure 1. Transition between normal-appearing appendiceal epithelium (right, indicated by green arrow) and transformed epithelium with lesions characteristic of low-grade appendiceal mucinous neoplasm (left), represented by reactive epithelium (red arrow), which has a muscular layer underneath (yellow arrow), and dysplastic epithelium (black arrow), underneath which the muscular layer of the mucosa is missing (white arrow), HE, 100×.

status of the invasion. When serosa is affected, portions of the hyaline wall are replaced by bands of low-grade mucinous epithelial cells that abundantly produce extracellular mucin. In some cases, mucin causes dissection of the appendix wall, resembling diverticula, and further ruptures of the appendix, with intraperitoneal seeding of mucinous tumor cells [6, 12].

Genetic features

In low-grade appendiceal mucinous neoplasms, mutations have been identified in many genes that are part of the RAS–RAF–MEK–ERK, PI3K–PKB/AKT, JAK–STAT, angiogenesis (including NOTCH) WNT and TGFB–TGFBR–SMAD signaling pathways (Figure 2). A smaller number of mutations are present in genes involved in DNA metabolism/expression, in genes encoding for enzymes and in genes with various cellular activities. In the RAS–RAF–MEK–ERK signaling pathway, genes mutated in low-grade appendiceal mucinous neoplasms are *KRAS*, *NRAS*, *BRAF*, mainly targeting the *KRAS* (Kirsten ras oncogene homolog) gene. It is part of the RAS gene family, which encodes GTPases that transduce signals between the cell membrane and the Golgi apparatus and are involved in proliferation, cell adhesion and migration, evading apoptosis, and stimulating angiogenesis. In some cases, mutations of the *KRAS* gene are present together with inactivating mutations of the *TP53* (Tumor Protein 53) gene. Another member of this gene family, *NRAS* (Neuroblastoma RAS Viral Oncogene Homolog), appears mutated in some cases of low-grade appendiceal mucinous neoplasms. Of the RAF

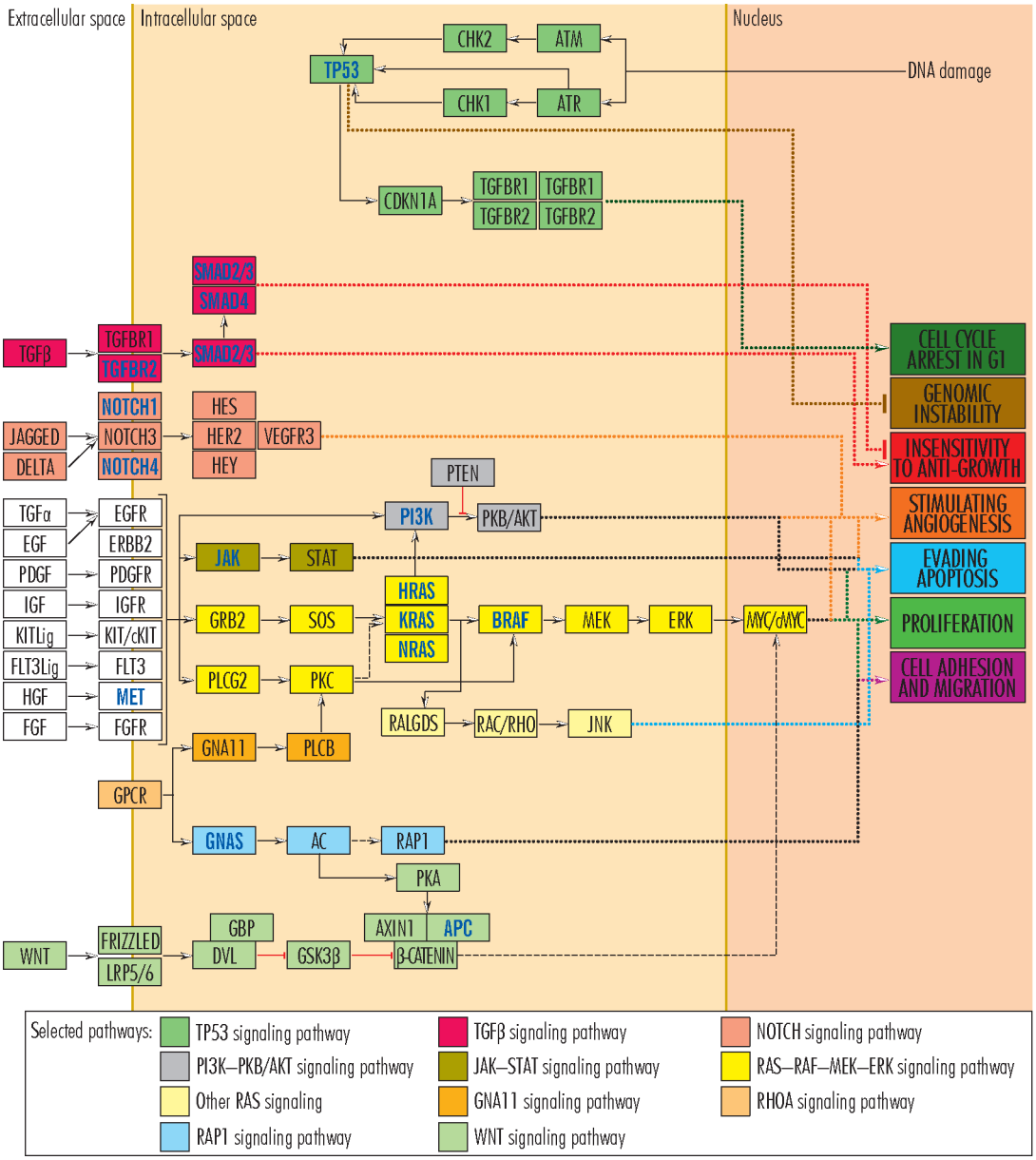


Figure 2. Altered signaling pathways in low-grade appendiceal mucinous neoplasms. Genes affected by mutations are written in bold, blue characters.

gene family, the *BRAF* (Rapidly accelerated fibrosarcoma B) gene is mutated, the most common defect affecting codon 600 (V600E), sometimes occurring simultaneously with mutations in the *TP53* gene. Of the genes involved in the PI3K–PKB/AKT signaling pathway, most mutations occur in the *PIK3CA* (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha), and *AKT1* (AKT Serine/Threonine Kinase 1) genes. The PI3K–PKB/AKT pathway constitutes an alternative pathway to RAS–RAF–MEK–ERK by which tumors proliferate, evade apoptosis, and stimulate angiogenesis. In most cases, mutations in the *PIK3CA* gene rarely occur simultaneously with those in *KRAS*. The JAK–STAT signaling pathway contributes to evading apoptosis and stimulating angiogenesis and, in low-grade appendiceal mucinous neoplasms, the most mutated gene is *JAK3* (Janus Kinase 3 protein). Stimulation of angiogenesis is enhanced by mutations in *GNAS* (G Protein Subunit Alpha S or Secretogranin VI) gene, which modulates the function of a number of hormones and molecules and is also involved in activating cAMP and stimulating several signaling pathways, and *CBP/CREBBP* (Cyclic adenosine monophosphate Response Element Binding Protein or CREB-binding protein) gene, which is a cofactor in the transcription of many genes, including *MYB*, *JUN*, *FOS*, *E1A* and *E6* oncogenes, as well as the tumor suppressor genes *TP53*, *E2F* (E2F Transcription Factor), *RB* (Retinoblastoma-Associated Protein), *SMAD*s (Mothers Against DPP Homologues), *RUNX*s (Runt-Related Transcription Factors) and *BRCA1* (Breast And Ovarian Cancer Susceptibility Protein 1). The most common mutations in the *GNAS* gene are p.R201H, c.602G>A and p.R201C, c.602 C>T. These defects are likely to play an important role in the mucin abundance that characterizes low-grade appendiceal mucinous neoplasms. The four members of the *NOTCH* gene family encode the type I transmembrane receptors NOTCH1 through NOTCH4 (Translocation-Associated Notch Protein TAN-1–4), which play an important role in the transduction of the pro-angiogenic signal via the NOTCH–HER/ERBB2–HES/HEY–VEGFR3 pathway. Inactivating mutations in the *APC* (Adenomatosis Polyposis Coli Tumor Suppressor) gene, which acts as an antagonist of the WNT signaling pathway, contribute to MYC/cMYC (V-Myc Avian Myelocytomatosis Viral Oncogene Homolog) activation and cell proliferation, tumor invasion and metastasis, evasion of apoptosis, and angiogenesis, all very important events in tumorigenesis. Insensitivity to anti-growth signals via the TGFB–TGFBR–SMAD signaling pathway sustains tumor growth. Of the genes involved in this signaling pathway, *TGFBR2* (Transforming Growth Factor Beta Receptor 2), *SMAD2* (SMAD-Mothers Against Decapentaplegic Homologue 2), *SMAD3* (SMAD-Mothers Against Decapentaplegic Homologue 3) and *SMAD4* (SMAD-Mothers Against Decapentaplegic Homologue 4) are mutated in a few cases of low-grade appendiceal mucinous neoplasms. Mutations in other genes, such as those involved in DNA metabolism/expression (*FANCA*, *RAD51C*), in those encoding for enzymes (*ARID1A*, *DIS3*, *FH*, *SMARCA4*) and in genes with various cellular activities (*FAT4*, *MED12*, *RNF43*, *STK11*, *TSC1*), are rare, but these may be important events in the tumor process [6, 13, 14, 15, 16, 17].

Staging and prognosis of low-grade appendiceal mucinous neoplasms

According to the Union for International Cancer Control (UICC) staging system, low-grade appendiceal mucinous neoplasms comprise stages pTis, pT3 and pT4, while pT1 and pT2 are missing. Thus, tumors extending only to the muscularis propria, without affecting the mesoappendix or serosa, are considered pTis, although this diagnosis requires correlation with the intraoperative findings and an evaluation by the operator (Table 1; Figure 3). Low-grade appendiceal mucinous neoplasms staged pTis are at no risk of recurrence and have an excellent prognosis. Tumors with acellular mucin or mucinous epithelium extending into the subserosa (without serosa involvement) or mesoappendix are considered pT3. To observe the extent of the tumor and

Table 1. Staging and prognosis of low-grade appendiceal mucinous neoplasms

pT stage	Features	Prognostic
pTis	Lesion affecting only the appendiceal wall, with the possibility of acellular mucin or mucinous epithelium disrupting muscularis propria. Histological examination of the entire appendix is required.	In general, there is no risk of recurrence.
pT3	Appendiceal subserosal or mesoappendix involvement without extension to the serosa. Histological examination of the entire appendix is required.	As the risk for recurrence is not known, follow-up is required for 10 years until the risk of recurrence is updated.
pT4a	Peritoneal involvement and invasion of the serosa or mesoappendix with cellular or acellular mucin	In acellular mucin, the risk of peritoneal recurrences is low. In the case of cellular mucin, the risk of peritoneal recurrences is high. Cytoreductive surgery with or without HIPEC, and follow-up within 10 years is required.
pT4b	Involvement of the peritoneum and direct invasion of adjacent organs or structures	

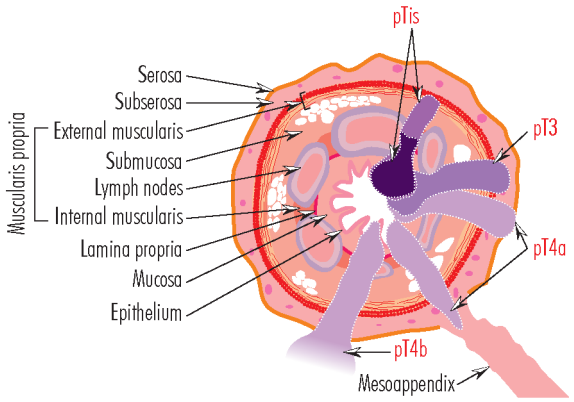


Figure 3. Illustration of structures affected by low-grade appendiceal mucinous neoplasms in different stages. With black text the normal components of the appendix wall are shown in cross-section and with red text the low-grade appendiceal mucinous neoplasms.

whether or not it affects the subserosa, histological examination of the entire appendix is required. Low-grade appendiceal mucinous neoplasms staged pT3 have an unknown risk of peritoneal recurrence, requiring long-term follow-up for 10 years or until the recurrence status changes. By invasion of at least the appendiceal serosa or by perforation of the appendix with invasion of adjacent organs, tumors are classified as pT4. In this sense, when mucinous epithelium invades the appendiceal serosa or mesoappendix, or when the appendiceal tumor seeds the peritoneal cavity and acellular/cellular mucin invades the visceral peritoneum, the tumors are staged as pT4a. The presence of mucin does not include luminal or mural spread in the cecum, but mucinous deposits on the serosa are associated with neovascularization, being traversed by small capillaries with red cells. The presence of neovascularization indicates activation of signaling pathways that promote angiogenesis. On the other hand, when cell seeding from the tumor into the peritoneal cavity leads to direct invasion of adjacent organs and structures, tumors are staged as pT4b. Peritoneal dissemination limited to acellular mucin only indicates stage M1a. When metastases are confined to the peritoneum only, regardless of their nature, the tumor stage is M1b, and when they develop outside the peritoneum, the tumor stage is M1c. The risk of peritoneal recurrence is reduced when the mucin is acellular, but becomes increased in the presence of cellular mucin, and long-term follow-up for 10 years with periodic imaging is highly required [16, 18].

Metastasis and complications of low-grade appendiceal mucinous neoplasms

Low-grade appendiceal mucinous neoplasms are recognized as indolent tumors, characterized by “push” patterns of growth instead of invasiveness, and which rarely metastasize or produce complications. Metastases derived from low-grade appendiceal mucinous neoplasms occur preferentially in the peritoneal cavity and ultimately lead to pseudomyxoma peritonei [1, 19, 20, 11, 4, 21], a life-threatening condition. Rarely, low-grade appendiceal mucinous neoplasms can metastasize to the fallopian tube mucosa [22], pulmonary pleura [23, 24] and inguinal nodes [25] (Figure 4). Although located at a great distance, pleuropulmonary metastases may arise from low-grade appendiceal mucinous neoplasms alone [23] or from their collisions with appendiceal neuroendocrine tu-

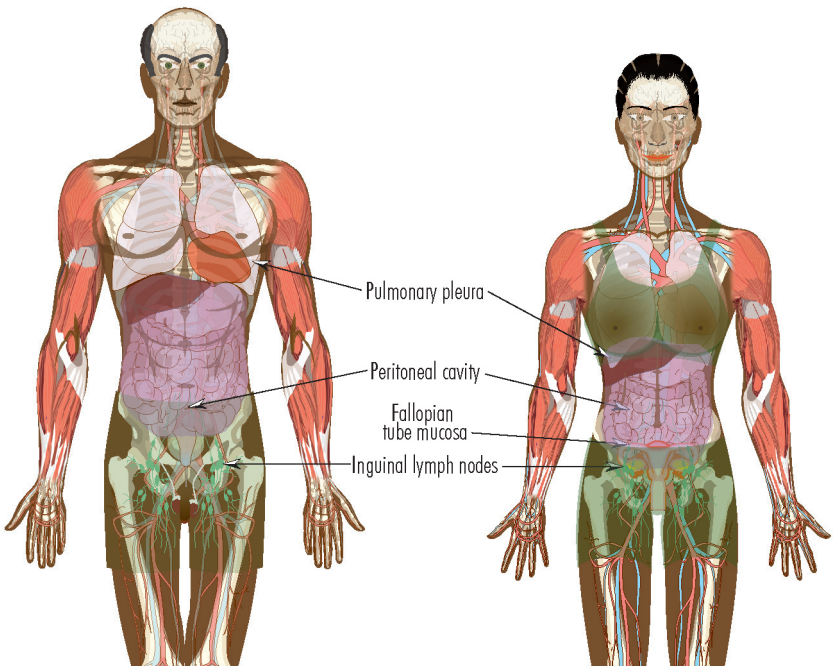


Figure 3. Metastatic sites of low-grade appendiceal mucinous neoplasms.

mors [24]. In some cases, low-grade appendiceal mucinous neoplasms may occur in association with other tumor times, such as urothelial carcinoma [26] and mucinous neoplasm of the renal pelvis [27], with no known causal relationship between them.

Among the most common complications of low-grade appendiceal mucinous neoplasms are ileocecal intussusception with bowel obstruction [28, 29], small bowel obstruction [30], ureteral obstruction [2], volvulus [31], ovarian lesions [32], rupture [33], abscesses [34], fistula [35, 36], and pseudomyxoma peritonei [2, 33]. Abscesses originating in low-grade appendiceal mucinous neoplasms can occur in internal organs in close proximity to the appendix, including the fallopian tube and ovary [37] and the iliopsoas muscle, the latter being very difficult to treat or untreatable. Appendiceal fistula defines spontaneous rupture of inflamed appendix internally, into urinary bladder, ileum, caecum, duodenum, ascending colon, Meckel's diverticulum and uterus, and externally, into right buttock, right flank, iliac fossa, groin or umbilicus [38]. Sometimes external fistulas may open from an internal abscess in contact with the tip of the neoplastic appendix. Fistula formation can lead to favorable prognosis. When the evacuation of appendix contents occurs in the peritoneal cavity and tumor cells seed the peritoneum, pseudomyxoma peritonei may result. Its frequency is 1-2 per million and it is a debilitating, disabling condition with increased risk of death [39, 40].

Treatment options

According to the Chicago Consensus Working Group [41], the first invasive investigation is surgical exploration to identify peritoneal spread. In low-grade appendiceal mucinous neoplasms, appendectomy is recommended, with verification of the invasiveness of the margins of the tumor lesion. When the margins are negative, the therapeutic course follows two strategies, depending on the perforation of the appendiceal wall. Thus, when the appendiceal wall is not perforated and the mucin or neoplastic cells have not been seeded extra-appendiceal, appendectomy is curative, without the need for post-operative monitoring. However, when mucin or mucinous cells are present extra-appendiceal, consideration of intraperitoneal chemotherapeutic treatment with subsequent monitoring is recommended. When the margins of the appendiceal tumor lesion are positive, cecectomy or ileocecectomy is recommended, with subsequent monitoring. For pseudomyxoma peritonei, cytoreductive surgery and hyperthermic intraperitoneal chemotherapy are indicated, with or without perioperative systemic chemotherapy [41, 42, 43, 44]. For low-grade appendiceal mucinous neoplasms, one-year survival is over 90% and five-year survival is over 80%, decreasing considerably for complicated cases, including pseudomyxoma peritoneum [45].

Discussions

Low-grade appendiceal mucinous neoplasms are epithelial tumors that result from uncontrolled proliferation of mucinous cells in the lining of the appendix. Mucin becomes accumulated as vacuoles in the cell cytoplasm. As the vacuoles increase in volume, they marginalize the cell nucleus, in some cases compressing it. At the same time, the wall of the appendix becomes fibrotic and hyalinized, gradually giving way to mucinous cells. It is important to note that the margins of low-grade appendiceal mucinous neoplasms advance by pushing, without invading the surrounding area. Sometimes, the pressure that the mucin exerts on the appendiceal wall is felt as pain and leads to the diagnosis of appendicitis, and the tumor progression is stopped by appendectomy. These are the happiest cases and are usually free of recurrences, with patients resuming their pre-operative lives. At other times, the pain is absent and the progression continues, with mucin accumulation and cell proliferation continuing and pressing on the wall of the appendix, which becomes very swollen and may rupture. Through the fissure, the contents of the appendix are released, consisting of acellular mucin or mucin with tumor cells, which reaches the peritoneal cavity and seeds various organs, most commonly the peritoneum or, in the case of women, the internal genitalia. There are cases when the ruptured appendix is trapped by internal organs (cecum, internal genitalia, bladder, iliopsoas muscle) or the abdominal wall and perforates them, producing abscesses or fistulas through which its contents are released. When the appendix is not attached to any internal organ, its contents are discharged into the abdominopelvic cavity. In the absence of pain, other symptoms do not alarm patients, and the diagnosis cannot easily be made. It is only when the fistula perforates the abdominal wall or when the accumulation of mucin in the abdomen causes it to become distended that patients receive a strong alarm signal and present themselves to the doctor. Through imaging investigations, surgery and pathological, genetic and immunohistochemical analyses, the correct diagnosis is made and patients are given a chance of a cure. While for some cases a single surgical intervention, followed or not by chemotherapy, is sufficient for cure, other cases are marked by recurrences, requiring repeated evacuation of mucinous accumulations, which, over time, can become disabling, difficult to operate and with an increased risk of death.

Conclusions

Low-grade appendiceal mucinous neoplasms are diseases with semi-malignant features which, when confined to the appendiceal mucosa, have a very good prognosis, but as they become more extensive and extend beyond the appendix, they become more difficult to treat and have a higher risk of recurrence and death. As rare neoplasms, no standardized methods of diagnosis, treatment or monitoring have been developed for low-grade appendiceal mucinous neoplasms, making their management difficult, especially for advanced cases.

Future perspectives

For low-grade appendiceal mucinous neoplasms, future perspectives are directed towards difficult cases, including pseudomyxoma peritonei, and are geared towards improving diagnosis, increasing treatment efficacy and reducing the risk of recurrence and mortality. To improve diagnosis, a battery of specific markers for these diseases needs to be identified and included in a set of analyses performed annually. To increase the effectiveness of treatment, knowledge of the disease and its response to therapy needs to be improved. In addition, it is necessary to test innovative therapies or to find therapeutic strategies that combine several types of treatment and that take into account the genetic characteristics of this type of tumor in order to reduce their resistance and the risk of recurrence as much as possible.

Conflicts of interest

The author declares that he has no conflicts of interest.

References

1. Misdraji J. Mucinous epithelial neoplasms of the appendix and pseudomyxoma peritonei. Mod Pathol. 2015 Jan;28 Suppl 1:S67-79. doi: 10.1038/modpathol.2014.129. PMID: 25560600.
2. Gonzalez HH, Herard K, Mijares MC. A Rare Case of Low-grade Appendiceal Mucinous Neoplasm: A Case Report. Cureus. 2019 Jan 29;11(1):e3980. doi: 10.7759/cureus.3980. PMID: 30967980; PMCID: PMC6440557.
3. Köhler F, Reese L, Hendricks A, Kastner C, Müller S, Lock JF, Germer CT, Wiegering A. Low-grade mucinous neoplasms (LAMN) of the appendix in Germany between 2011 and 2018: a nationwide analysis based on data provided by the German Center for Cancer Registry Data (ZfKD) at the Robert Koch Institute (RKI). Langenbecks Arch Surg. 2022 Dec;407(8):3615-3622. doi: 10.1007/s00423-022-02639-w. Epub 2022 Aug 12. PMID: 35962281; PMCID: PMC9722888.
4. Akay E, Arslan A, Eren SK, Özhan N, Karaman H. Mucinous appendiceal neoplasms and pseudomyxoma peritonei: Morphological and clinical findings, differential diagnosis, and prognosis. Indian J Pathol Microbiol. 2022 Jul-Sep;65(3):565-571. doi: 10.4103/ijpm.ijpm_827_21. PMID: 35900483.
5. Xu R, Yang ZL. Treatment of a giant low-grade appendiceal mucinous neoplasm: A case report. World J Clin Cases. 2021 Dec 16;9(35):11056-11060. doi: 10.12998/wjcc.v9.i35.11056. PMID: 35047618; PMCID: PMC8678861.
6. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA. WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020 Jan;76(2):182-188. doi: 10.1111/his.13975. Epub 2019 Nov 13. PMID: 31433515; PMCID: PMC7003895.
7. Constantin M, Petrescu L, Mătanie C, Vrancianu C), Niculescu A-G, Andronic O, Bolocan A. The Vermiform Appendix and Its Pathologies. Cancers. 2023; 15(15):3872. doi: 10.3390/cancers15153872
8. Bell PD, Huber AR, Drage MG, Barron SL, Findeis-Hosey JJ, Gonzalez RS. Clinicopathologic Features of Low-grade Appendiceal Mucinous Neoplasm: A Single-institution Experience of 117 Cases. Am J Surg Pathol. 2020 Nov;44(11):1549-1555. doi: 10.1097/PAS.0000000000001551. PMID: 32796173.
9. Pai RK, Beck AH, Norton JA, Longacre TA. Appendiceal mucinous neoplasms: clinicopathologic study of 116 cases with analysis of factors predicting recurrence. Am J Surg Pathol. 2009 Oct;33(10):1425-39. doi: 10.1097/PAS.0b013e3181af6067. PMID: 19641451.
10. Misdraji J, Yantiss RK, Graeme-Cook FM, Balis UJ, Young RH. Appendiceal mucinous neoplasms: a clinicopathologic analysis of 107 cases. Am J Surg Pathol. 2003 Aug;27(8):1089-103. doi: 10.1097/00000478-200308000-00006. PMID: 12883241.
11. Carr NJ, McCarthy WF, Sobin LH. Epithelial noncarcinoid tumors and tumor-like lesions of the appendix. A clinicopathologic study of 184 patients with a multivariate analysis of prognostic factors. Cancer. 1995 Feb 1;75(3):757-68. doi: 10.1002/1097-0142(19950201)75:3<757::aid-cncr2820750303>3.0.co;2-f. PMID: 7828125.
12. Cristian DA, Grama FA, Becheanu G, Pop A, Popa I, Şurlin V, Stănilescu S, Bratu AM, Burcoş T. Low-grade appendiceal mucinous neoplasm mimicking an adnexal

mass. Rom J Morphol Embryol. 2015;56(2 Suppl):837-42. PMID: 26429182.

13. Munari G, Businello G, Mattiolo P, Pennelli G, Sbaraglia M, Borga C, Pucciarelli S, Spolverato G, Mescoli C, Galuppini F, Sommariva A, Bellan E, Lonardi S, Loupakis F, Luchini C, Dei Tos AP, Fassan M. Molecular profiling of appendiceal serrated lesions, polyps and mucinous neoplasms: a single-centre experience. J Cancer Res Clin Oncol. 2021 Jul;147(7):1897-1904. doi: 10.1007/s00432-021-03589-4. Epub 2021 Mar 12. PMID: 33712927; PMCID: PMC8164605.
14. Liu X, Mody K, De Abreu FB, Pipas JM, Peterson JD, Gallagher TL, Suriawinata AA, Ripple GH, Hourdequin KC, Smith KD, Barth RJ Jr, Colacchio TA, Tsapakos MJ, Zaki BI, Gardner TB, Gordon SR, Amos CI, Wells WA, Tsongalis GJ. Molecular profiling of appendiceal epithelial tumors using massively parallel sequencing to identify somatic mutations. Clin Chem. 2014 Jul;60(7):1004-11. doi: 10.1373/clinchem.2014.225565. Epub 2014 May 12. PMID: 24821835.
15. Pengelly RJ, Rowaiye B, Pickard K, Moran B, Dayal S, Tapper W, Mirnezami A, Cecil T, Mohamed F, Carr N, Ennis S. Analysis of Mutation and Loss of Heterozygosity by Whole-Exome Sequencing Yields Insights into Pseudomyxoma Peritonei. J Mol Diagn. 2018 Sep;20(5):635-642. doi: 10.1016/j.jmoldx.2018.05.002. Epub 2018 Jun 22. PMID: 29936255.
16. Constantin M, Mătanie C, Petrescu L, Bolocan A, Andronic O, Bleotu C, Mitache MM, Tudorache S, Vrancianu CO. Landscape of Genetic Mutations in Appendiceal Cancers. Cancers. 2023; 15(14):3591. https://doi.org/10.3390/cancers15143591.
17. Alakus H, Babicky ML, Ghosh P, Yost S, Jepsen K, Dai Y, Arias A, Samuels ML, Mose ES, Schwab RB, Peterson MR, Lowy AM, Frazer KA, Harismendy O. Genome-wide mutational landscape of mucinous carcinomatosis peritonei of appendiceal origin. Genome Med. 2014 May 29;6(5):43. doi: 10.1186/gm559. PMID: 24944587; PMCID: PMC4062050.
18. Kang DW, Kim BH, Kim JM, Kim J, Chang HJ, Chang MS, Sohn JH, Cho MY, Jin SY, Chang HK, Han HS, Kim JY, Kim HS, Park DY, Park HY, Lee SJ, Lee W, Lee HS, Kang YN, Choi Y. Gastrointestinal Pathology Study Group of the Korean Society of Pathologists. Standardization of the pathologic diagnosis of appendiceal mucinous neoplasms. J Pathol Transl Med. 2021 Jul;55(4):247-264. doi: 10.4132/jptm.2021.05.28. Epub 2021 Jul 8. PMID: 34233112; PMCID: PMC8353140.
19. Foster JM, Sleightholm RL, Wahlmeier S, Loggie B, Sharma P, Patel A. Early identification of DPAM in at-risk low-grade appendiceal mucinous neoplasm patients: a new approach to surveillance for peritoneal metastasis. World J Surg Oncol. 2016 Sep 13;14(1):243. doi: 10.1186/s12957-016-0996-0. PMID: 27619776; PMCID: PMC5020442.
20. Ramaswamy V. Pathology of Mucinous Appendiceal Tumors and Pseudomyxoma Peritonei. Indian J Surg Oncol. 2016 Jun;7(2):258-67. doi: 10.1007/s13193-016-0516-2. Epub 2016 Mar 19. PMID: 27065718; PMCID: PMC4818623.
21. Hayler R, Lockhart K, Barat S, Cheng E, Mui J, Shamavonian R, Ahmadi N, Alzahrani N, Liauw W, Morris D. Survival benefits with EPIC in addition to HIPEC for low grade appendiceal neoplasms with pseudomyxoma peritonei: a propensity score matched study. Pleura Peritoneum. 2023 Mar 16;8(1):27-35. doi: 10.1515/pp-2022-0205. PMID: 37020474; PMCID: PMC10067551.
22. Siatecka H, Al-Awami S, Masand RP. Isolated Metastasis to Fallopian Tube Mucosa by Low-Grade Appendiceal Mucinous Neoplasm: Report of Two Cases. Int J Surg Pathol. 2023 Aug;31(5):646-650. doi: 10.1177/10668969221117980. Epub 2022 Aug 9. PMID: 35946118.
23. Zhao XY, Li Cq, Zhang SY, Liu G. Case Report: An Unusual Case of Pulmonary Metastatic Adenocarcinoma From Low-Grade Appendiceal Mucinous Neoplasms. Front Oncol. 2022 Jul 13;12:906344. doi: 10.3389/fonc.2022.906344. PMID: 35912193; PMCID: PMC9327614.
24. Gao J, Fan R, Lu M. Pulmonary pleura metastasis from low-grade appendiceal mucinous neoplasm with appendiceal neuroendocrine tumor: A Case Report. Research Square.2022. doi: 10.21203/rs.3.rs-1549098/v1.
25. Vaz-Pereira R, Marques R, Fernandes U, Monteiro A, Pinto-De-Sousa J. Mucinous adenocarcinoma of the appendix with inguinal node metastases. Autops Case Rep [Internet]. 2021;11:e2021335. https://doi.org/10.4322/acr.2021.335.
26. Al-Janabi MH, Zidan T, Zidan E, Muhammed MS, Salloum R. Low-grade appendiceal mucinous neoplasm associated with Urothelial carcinoma: A rare case report from Syria. Ann Med Surg (Lond). 2022 Mar 29;76:103525. doi: 10.1016/j.amsu.2022.103525. PMID: 35495395; PMCID: PMC9052276.
27. Zou Y, Xie X, Wang Q, Zhong C, Liu Q. Case report: A rare case of synchronous mucinous neoplasms of the renal pelvis and the appendix. Front Oncol. 2023 Jun

26;13:1213631. doi: 10.3389/fonc.2023.1213631. PMID: 37434974; PMCID: PMC10331127.

28. Liapis SC, Perivoliotis K, Psarianos K, Chatzinikolaou C, Moula AI, Skoufogiannis P, Balogiannis I, Lytras D. A giant low-grade appendiceal mucinous neoplasm (LAMN) presenting as ileocecal intussusception: a case report. J Surg Case Rep. 2023 May 27;2023(5):rjad273. doi: 10.1093/jscr/rjad273. PMID: 37251252; PMCID: PMC10224788.
29. Houlzé-Laroye C, Eveno C. Low-grade appendiceal mucinous neoplasms with bowel obstruction. Pleura Peritoneum. 2019 Oct 11;4(3):20190020. doi: 10.1515/pp-2019-0020. PMID: 31667332; PMCID: PMC6812216.
30. Komo T, Kohashi T, Hihara J, Oishi K, Yoshimitsu M, Kanou M, Nakashima A, Aoki Y, Miguchi M, Kaneko M, Mukaida H, Hirabayashi N. Intestinal obstruction caused by low-grade appendiceal mucinous neoplasm: A case report and review of the literature. Int J Surg Case Rep. 2018;51:37-40. doi: 10.1016/j.ijscr.2018.08.001. Epub 2018 Aug 9. PMID: 30138868; PMCID: PMC6104576.
31. Costa V, Demuro JP. Low-grade appendiceal neoplasm presenting as a volvulus of the cecum. Gastroenterol Rep (Oxf). 2013 Nov;1(3):207-10. doi: 10.1093/gastro/got026. Epub 2013 Oct 8. PMID: 24759968; PMCID: PMC3937993.
32. Karande GY, Chua WM, Yiin RSZ, Wong KM, Hedgire S, Tan TJ. Spectrum of computed tomography manifestations of appendiceal neoplasms: acute appendicitis and beyond. Singapore Med J. 2019 Apr;60(4):173-182. doi: 10.11622/smedj.2019035. PMID: 31069398; PMCID: PMC6482419.
33. Wang AS, Ismael HN, Parikh J, Modesto VL. Low-Grade Appendiceal Mucinous Neoplasm: A Case Series. Cureus. 2022 Sep 3;14(9):e28755. doi: 10.7759/cureus.28755. PMID: 36211097; PMCID: PMC9529292.
34. Huang X, Xiao Z, Huang Z. Huge Abdominal Abscess Secondary to an Appendiceal Mucinous Neoplasm. Clin Gastroenterol Hepatol. 2020 May;18(5):e62. doi: 10.1016/j.cgh.2019.03.031. Epub 2019 Mar 27. PMID: 30928455.
35. Aung S, Cheung A, Prather C, Lai J. Diarrhea Concealing a Duodenal-Cecal Fistula Secondary to Appendiceal Mucinous Neoplasm. ACG Case Rep J. 2017 Jan 4;4:e3. doi: 10.14309/crj.2017.3. PMID: 28138447; PMCID: PMC5244891.
36. Cohen R, Mckean K, Chetrit S. Enterocutaneous fistula resulting from the drainage of a contained appendiceal perforation secondary to a partially obstructing low-grade appendiceal mucinous neoplasm. BMJ Case Reports CP. 2021;14:e244546.
37. Cubro H, Cengic V, Burina N, Kravic Z, Beciragic E, Vranic S. Mucocele of the appendix presenting as an exacerbated chronic tubo-ovarian abscess: A case report and comprehensive review of the literature. Medicine (Baltimore). 2019 Sep;98(39):e17149. doi: 10.1097/MD.0000000000017149. PMID: 31574819; PMCID: PMC6775329.
38. Singh MP, Samaiya A, Sainia TK, Saxena A. Spontaneous appendico-cutaneous fistula: an unusual presentation of retroperitoneal appendicular perforation associated with psoas abscess. J Surg Case Rep. 2016 Jun 23;2016(6):rjw112. doi: 10.1093/jscr/rjw112. PMID: 27340228; PMCID: PMC4918459.
39. Amini A, Masoumi-Moghaddam S, Ehteda A, Morris DL. Secreted mucins in pseudomyxoma peritonei: pathophysiological significance and potential therapeutic prospects. Orphanet J Rare Dis. 2014 May 5;9:71. doi: 10.1186/1750-1172-9-71. PMID: 24886459; PMCID: PMC4013295.
40. Gleeson EM, Feldman R, Mapow BL, Mackovick LT, Ward KM, Morano WF, Rubin RR, Bowne WB. Appendix-derived Pseudomyxoma Peritonei (PMP): Molecular Profiling Toward Treatment of a Rare Malignancy. Am J Clin Oncol. 2018 Aug;41(8):777-783. doi: 10.1097/COC.0000000000000376. PMID: 28263231. Am J Clin Oncol. 2018 Aug;41(8):777-783. doi: 10.1097/COC.0000000000000376. PMID: 28263231.
41. Chicago Consensus Working Group. The Chicago Consensus on peritoneal surface malignancies: Management of appendiceal neoplasms. Cancer. 2020 Jun 1;126(11):2525-2533. doi: 10.1002/cncr.32881. Epub 2020 Apr 13. PMID: 32282073.
42. Lin YL, Xu DZ, Li XB, Yan FC, Xu HB, Peng Z, Li Y. Consensuses and controversies on pseudomyxoma peritonei: a review of the published consensus statements and guidelines. Orphanet J Rare Dis. 2021 Feb 13;16(1):85. doi: 10.1186/s13023-021-01723-6. PMID: 33581733; PMCID: PMC7881689. Orphanet J Rare Dis. 2021 Feb 13;16(1):85. doi: 10.1186/s13023-021-01723-6. PMID: 33581733; PMCID: PMC7881689.
43. Bijelic L, Kumar AS, Stuart OA, Sugarbaker PH. Systemic Chemotherapy prior to Cytoreductive Surgery and HIPEC for Carcinomatosis from Appendix Cancer: Impact on Perioperative Outcomes and Short-Term Survival. Gastroenterol Res Pract. 2012;2012:163284.

doi: 10.1155/2012/163284. Epub 2012 Jul 26. PMID: 22899903; PMCID: PMC3412098.

44. Blackham AU, Swett K, Eng C, Sirintrapun J, Bergman S, Geisinger KR, Votanopoulos K, Stewart JH, Shen P, Levine EA. Perioperative systemic chemotherapy for appendiceal mucinous carcinoma peritonei treated with cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. J Surg Oncol. 2014 Jun;109(7):740-5. doi: 10.1002/jso.23547. Epub 2013 Dec 28. PMID: 24375188; PMCID: PMC4010799.
45. Gok M, Topal U, Akyüz M, Öz AB, Sozuer E, Deniz K. Long-term Results of Low Grade Appendiceal Mucinous Neoplasm (LAMN): A Retrospective Analysis of 24 Patients. Arch Iran Med. 2021 Aug 1;24(8):615-621. doi: 10.34172/aim.2021.87. PMID: 34488329.