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Comparative Spectral Analysis of *Octopus vulgaris* Cuvier, 1797, Jellyfish and Seawater from Aegean Sea, Evia, Greece

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Research was conducted using the Fourier Transform Infrared (FTIR) spectral spectroscopy method on octopus, jellyfish, and seawater from the Aegean Sea, Chalkida, Evia Island, Greece. Our analyses of two peaks at 896 and 933 cm^{-1} indicate their presence in the octopus. These peaks are expressed in Martian minerals and meteorites. The results suggest that the octopus is adaptable to conditions on another planet. Our data provide a basis for speculating that it is an organism from Mars or another planet with similar characteristics. However, the spectral characteristics of the jellyfish and seawater do not correspond to the spectral characteristics of Martian minerals and meteorites. Studies have been conducted also on the microbial flora on the

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skin of octopus of the species *Octopus vulgaris* Cuvier, 1797, captured in the Aegean Sea near Chalkida, Greece, on the island of Euboea (Evia). The aim was to assess their role as carriers of pathogenic microorganisms. Bacteria from the families *Micrococcaceae* and *Enterobacteriaceae* have been isolated, as well as *Aeromonas hydrophilia ssp. hydrophilia*, *Enterococcus caseiflavus*, and *Candida lusitaniae*. *Staphylococci prevail*, with the highest quantity being *Staphylococcus simulans* (107.67 + 10.21 CFU cm^{-2} on the head and 67.33 + 6.02 CFUcm^{-2} on the tentacles), followed by *Staphylococcus piscifermentans* (14.33+1.69 CFU cm^{-2} on the head and 37.33+1.25 CFU cm^{-2} on the tentacles) and *Staphylococcus hyicus* (3.67+1.25 CFU cm^{-2} on the head and 14.67 + 3.39 CFU cm^{-2} on the tentacles). Among the Gram-negative bacteria, *Aeromonas hydrophilia ssp. hydrophilia piscifermentans* predominated, found only on the head of octopuses at 9.67+2.05 CFU cm^{-2}, followed by *Klebsiella pneumoniae ssp. ozaenae*, also isolated solely from the head (4.00+1.63 CFU cm^{-2}). *Escherichia coli* was the least prevalent, but it was detected both on the head (2.67+0.47 CFU cm^{-2}) and the tentacles of the studied animals (1.67+0.47 CFU cm^{-2}). Enterococci and fungi were isolated in small quantities from the octopuses' heads and tentacles. The isolated bacteria demonstrated in *vitro* resistance to most of the tested antibiotics. They were only sensitive to broad-spectrum agents such as amphenicols, tetracyclines, aminoglycoside-aminocyclitols (excluding kanamycin), and quinolones. Staphylococci also showed sensitivity to some penicillins (ampicillin, amoxicillin). However, lincosamides (clindamycin) were ineffective *in vitro* against the studied bacteria. Potentiated sulfonamides exhibited high activity against the Gram-negative bacteria but not against Gram-positive ones.*Aeromonas hydrophila* is known to be pathogenic to marine inhabitants, including octopuses. *S. simulans*, *E. coli*, and *K. pneumoniae* are conditionally pathogenic to animals and humans. Their presence on the surface of the studied octopuses, especially *E. coli*, could indicate seawater fecal contamination and possibly other pathogenic species. The current results also reveal the distribution of multidrug-resistant bacterial strains, even among bacteria that are representatives of the normal microflora of octopuses.

Keywords: Octopus; jellyfish; seawater; spectral analysis; microflora; pathogens; antibiotic resistance.

1. INTRODUCTION

Octopuses are intriguing and enigmatic and represent marine fauna that have been insufficiently studied. They belong to the class of cephalopod mollusks. There are 150 known species of octopuses worldwide. The common octopus, *Octopus vulgaris* Cuvier, 1797, is one of the most widespread globally. It is mainly found in the Aegean and Mediterranean Seas and the Atlantic and Pacific Oceans. Today, it is considered a species complex comprising six species, with *O. vulgaris* sensu stricto occurring in the Mediterranean Sea and the northeastern Atlantic Ocean [1-3]. *O. vulgaris* inhabits shallow rocky or sandy areas. During the day, it hides in dens. At night, it becomes active, moving short distances. Multiple paternity is characteristic during its reproduction. The female mates multiple times and takes care of the fertilized 100000–500000 eggs, refraining from feeding during this period. She dies after the hatching of her offspring. The embryonic development and growth of *O. vulgaris* occur within temperature ranges of 7–33°C. Their lifespan is short, about one year [2]. This brevity is strange for such intelligent and unique creatures as octopuses.

The population of *O. vulgaris* is highly sensitive to environmental factors, especially during the larval stage, which is why its quantity is diminishing. This is a worrisome trend associated with human activity, climate change, and an increase in their catches in recent years [2, 3]. Many octopuses fall prey to marine predators [4]. The decline in their population in Southern Europe is also primarily due to the growing interest in hunting these animals, driven by significant decreases in fish stocks and rising prices. Today, Morocco and Mauritania are the leading exporters of octopuses [5, 6].

Octopuses differ significantly from other creatures on our planet, with the presence of three hearts being particularly intriguing [7]. The eye structure is also different in octopuses. It is thought to have evolved by convergence in both fish and coleoid cephalopods (octopus, squid, and cuttlefish) but less developed in cubomedusan jellyfish to replace the curved cornea with a spherical lens with a graduated refractive index. The gradient goes from a water-like wavelength of light n=1.33 at the outer surface to a high n=1.60 at the core. Light passing through this increasing difference in refractive index is bent to a sharp focus on the retina, which is not as sharp in jellyfish [8].

Diseases in these animals also affect the population numbers, but they are still poorly studied. Farto et al. examined bacteria-causing diseases in *O. vulgaris* [9]. They have established that *Vibrio lentus* is a causative agent of infection in them, resulting in skin lesions, colonization of internal organs, and mortality. They also highlighted other genera of bacteria, including *Vibrio, Pseudomonas, Aeromonas (A. hydrophila, A. caviae), Cytophaga, Flavobacterium*, and *Klebsiella pneumoniae* as pathogens for octopuses.

Studies on the normal microflora of octopuses are limited. The gastrointestinal microbiome of wild paralarvae of *O. vulgaris* in the Mediterranean Sea has been investigated, revealing the dominance of two bacterial families – *Mycoplasmataceae* and *Vibrionaceae*. Gram-negative species from the *Bacteroidetes* phylum predominate in the octopus microflora in August, while those from *Proteobacteria* dominate in November [10].*Vibrio vulnificus* is reported as a causative agent of infection in octopuses. In South Korea, octopus is often consumed without thermal processing, posing a risk of human foodborne infections [10].

The intensive human industrial and agricultural activities lead to pollution of natural and artificial water bodies, primarily from sewage and direct contamination from wild and domestic animals, feed, and more. This has adverse ecological consequences on the health of aquatic inhabitants and, consequently, the environment. Moreover, it affects the safety of consuming fish and other aquatic foods, which could lead to human illnesses. From this perspective, studies on the presence and distribution of pathogens in marine organisms are significant to ensure their consumption safety and preserve the environment. Microbial assessments of aquatic inhabitants also provide additional information about the hygienic status of the environment, including seas, oceans, lakes, rivers, reservoirs, and fish farms. Detecting pathogenic microorganisms or changes in the natural microflora of aquatic inhabitants serves as an essential indicator of potential water contamination. Knowledge about these pathogens and current data on them is crucial for improving aquaculture management [9, 11] and for natural biodiversity conservation.

These findings directed our focus toward the objective of the current research, which aims to determine the presence of microorganisms from major groups with pathogenic potential on the skin of *Octopus vulgaris* inhabiting the Aegean Sea near the coast of Chalkida, Greece.To enhance our understanding of octopuses as unique organisms, we set out to conduct spectral analysis on octopus, jellyfish, and seawater from Chalkida, Evia Island, Greece.

2. MATERIALS AND METHODS

2.1 Fourier Transform Infrared Spectroscopy

IR-spectraof sea salt, jellyfish, and *O. vulgaris* from the Aegean sea was registered on Fourier-IR spectrometer Brucker Vertex ("Brucker", Germany) (a spectral range: average IR – 370 ÷ 7800 cm^{-1}; visible – 2500 ÷ 8000 cm^{-1}; the permission – 0.5 cm^{-1}; accuracy of wave number – 0.1 cm^{-1} on 2000 cm^{-1}); Thermo Nicolet Avatar 360 Fourier-transform IR.

2.2 Studied Sea Water and Animals

FTIR spectral analysis was conducted onseawater (Fig. 1), jellyfish (Fig.2), and octopus (Fig. 3) from Chalkida, Evia Island, Greece.

Fig. 1. The Aegean Sea near the coast of Chalkida

Fig. 2. Jellyfish captured in the Aegean Sea near the coast of Chalkida

Fig.3. *Octopus vulgaris* Cuvier, 1797, captured in the Aegean Sea near the coast of Chalkida

A study on osmosis and diffusion processes between 0.9% NaCl and deionized water was conducted by Mehandjiev et al.in 2023 [12].

Microbial flora studies were conducted on the surface of octopus of the species *Octopus vulgaris* Cuvier, 1797, captured in the Aegean Sea near the coast of Chalkida (Fig.3). It was purchased chilled from a commercial establishment and stored at -20°C until the research was conducted.

2.3 Culture Media

For the isolation of microorganisms, elective and selective culture media were used, including Mueller Hinton agar and broth, Colorex Chromogenic Orientation agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India), Chapman agar, Endo agar, Eosin Methylene Blue (EMB) agar, Sabouraud agar with chloramphenicol, and Columbia blood agar (Bul-Bio National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria).

2.4 Microbiological Investigations

Microbiological investigations were conducted using impression cultures by pressing the surface of the studied animals onto separate sectors within Petri dishes containing each of the selective solid media, with an area of 20 cm^2. The cultures were done separately for the octopus' head and tentacles. After incubation under aerobic conditions at 37°C for 24-72 hours, the formed colonies were counted and presented as colony-forming units per cm^2 ($CFUcm^{-2}$) of the examined skin surfaces.

2.5 Microscopic Examinations

Microscopic examinations of microorganisms were conducted under immersion at a magnification of 1000x after staining using classical methods such as Gram, Pfeiffer, Klett, and Mueller on microscopic slides prepared from the studied materials. Additionally, samples from cultures on various culture media were examined. A digital microscope with a camera, model B-190TV from Optika, Italy, was utilized.

2.6 Agglutination Reaction

The agglutination reaction on microscope slides for serogroup typing of isolated *E. coli* strains was performed using polyvalent agglutinating sera from groups I, II, and III (Bul-Bio NCIPD – Sofia, Bulgaria).

2.7 Taxonomic Identification

All clinical isolates were identified through conventional methods according to the 9th edition of Bergey's Manual [13]. The isolated microorganisms were identified through microscopic examination of differently stained slides. Their cultural and hemolytic properties were assessed on solid and liquid media. The biochemical characteristics were studied using biochemical identification tests for staphylococci, streptococci, and enterococci (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Additional tests were made for oxidase, catalase, etc., with reagents from Antisel (SharlauChemie S.A., Spain). Staphylococcal identification was performed using MicrolatestStaphytest 24, streptococcal identification - with MicrolatestStreptotest 24, Gram-negative bacteria - with MicrolatestEnterotest 24 N, and yeasts - with Microlatest Candida-Screen test 24. As per the test instructions, suspensions with a density of 2 according to the Mac Farland standard were prepared and dispensed by 0.1 ml into the respective wells of the test plates. Results were recorded after incubation at 37°C for 24 hours. Determination of the species affiliation of tested microorganisms was done using the keys provided with the tests after determining the codes of the isolated strains and finding them in the code books.

2.8 Quantitative Determination

Microorganisms were determined by counting the developed colonies and their mean arithmetic count and calculating the quantity of colony-forming units (CFU) per 1 cm^2 of the examined skin surfaces.

2.9 Determination of the Sensitivity of the Antimicrobial Agents

All clinical isolates' sensitivity to antimicrobial agents was determined using the classical agar disk diffusion method described by Bauer et al. [14]. Standard antibiotic susceptibility disks (Bul-Bio NCIPD – Sofia) and our prepared disks were used. Bacterial suspensions in the exponential growth phase with a concentration of 2.10^6 $cells.mL^{-1}$, determined using the optical standard of Mac Farland, were inoculated onto Columbia blood agar (Bul-Bio NCIPD – Sofia, Bulgaria) or Mueller-Hinton agar (Antisel - Sharlau Chemie S.A., Spain). Incubation was performed at 37°C for 24 hours. Results were recorded by measuring the diameters of the sterile zones in millimeters, including the diameter of the disk, with an accuracy of 1 mm. The zone's boundary was considered the point of complete growth inhibition. The results were interpreted using the three-tiered system by Bauer et al. [14] after measuring the diameters of the inhibitory zones in millimeters.

2.10 Statistical Analysis

This was performed by one-way analysis of variance (ANOVA), followed by Dunnett post-hoc test. The classic Student-Fisher method was also used.

3. RESULTS

3.1 Chemical Composition of Seawater from the Aegean Sea. Osmosis

Osmotic processes occur at the interface between two media with different concentrations. The transfer of electric charges, electric current, and conductivity was observed. It was found that the transport of electric charges involves Na^+, Cl^-, and H^+ ions [12]. As per the scheme by Mehandjiev et al. [12], an osmotic process was studied from seawater in the Aegean Sea to the island of Evia. The second water source was freshwater. The following results were obtained for Na^+, Cl^-, and pH.

It seems like you're discussing research on osmotic processes involving the transfer of ions such as Na^+ and Cl^- between seawater and freshwater, as well as the measurement of pH levels, electric current (I), voltage (U), and oxidation-reduction potential (ORP) in mV (Table 1).

In the investigations using the FTIR spectral analysis method on octopus, jellyfish, and seawater from Chalkida, Evia Island, Greece, the following results were obtained as wavenumbers within the atmospheric transparency window of the Earth from 8 to 14 μm (1250-714 cm^{-1}).

The data of the spectral analysisare visible from Fig. 4, 5, and 6 of the peaks in the IR spectrum

The wavenumbers within the atmospheric transparency window were:

Octopus
613; 656; 700; 896; 933; 1044; 1080; 1171 cm^{-1}
Jelly Fish
616; 701; 982; 1114 cm^{-1}
Sea Salt
618, 701, 879, 1117 cm^{-1}
The peaks in the range 1407-3548 $cm^{-1}$are the following:
Octopus
1454, 1541, 1651, 2124; 2874, 2927, 2961, 3290, 3414 cm^{-1}
Jelly Fish
1407, 1454, 1549, 1644, 2061, 2088, 3221, 3445 cm^{-1}
Sea Salt
1425; 1448; 1463; 1482; 1636; 1734; 3230; 3442 cm^{-1}

Table 1. Results with Sea water and fresh water during the osmosis process

	Na ($mg.L^{-1}$) Aegean Sea	Cl ($mg.L^{-1}$) Aegean Sea	pH	U (mV)	I (mA)	ORP (mV)
Control sample Aegean Sea water	10830 ±1083	16705 ±1671	7.58			221
Sample after osmosis	3860 ±386	5670 ±567	7.97	340	0.07	156

The power of the osmotic signal was $W=I.U=0.07x10^{-3}\ x0.34x10^{-3}=0.238.\ 10^{-6}\ W$

Let's calculate the area of the osmotic process

$S=2\pi rh=2x3.14x3x7=130\ cm^2$

$P=0.238.\ 10^{-6}\ /130=0.0018.10^{-6} W/cm^2=1.8.10^{-9}\ W/cm^2$

This electric signal has physiological effects on living organisms.

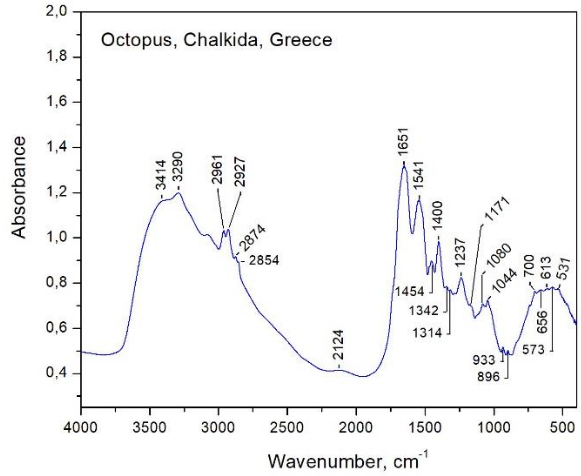

Fig. 4. Spectral analysis of *O. vulgaris*

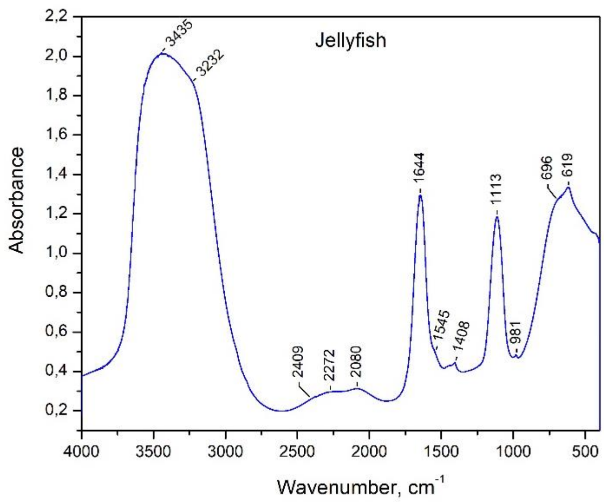

Fig. 5. Spectral analysis of jellyfish

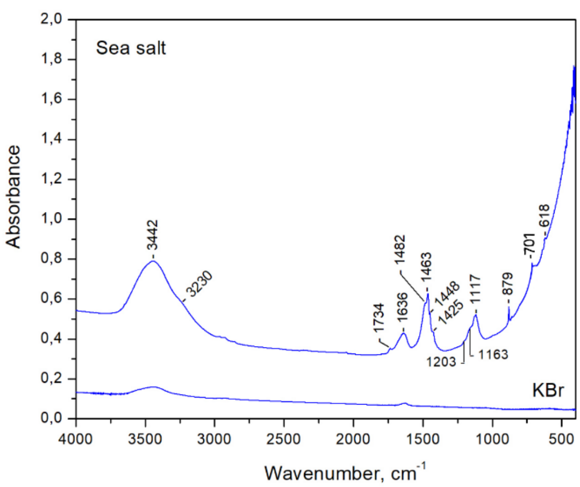

Fig. 6. Spectral analysis of sea salt

The data showed typical peaks among the octopus, jellyfish, and seawater spectra at 613-616, 700-701 cm^{-1}. Seawater, jellyfish, and octopus initially shared a 613-616 cm^{-1} range. Interestingly, typical peaks at 982 and 1114 cm^{-1} were observed in jellyfish and seawater.

The results indicate that the following wavenumbers are specific to the octopus – 2124,2874, 2927, 2961, 3290, 3414 cm^{-1}. Interestingly, these wavenumbers fall within the near and middle infrared range of 2.93 to 4.71 µm.

For sea salt and jellyfish, we identified the following common wavenumbers:
Jelly Fish
1454, 1644, 3221, 3445 cm^{-1}
Sea Salt
1448; 1636; 3230; 3442 cm^{-1}

Fig. 7 shows that around 4 µm, the spectrum of octopus absorption was higher than in the 8-14 µm range and had more peaks than seawater and jellyfish.

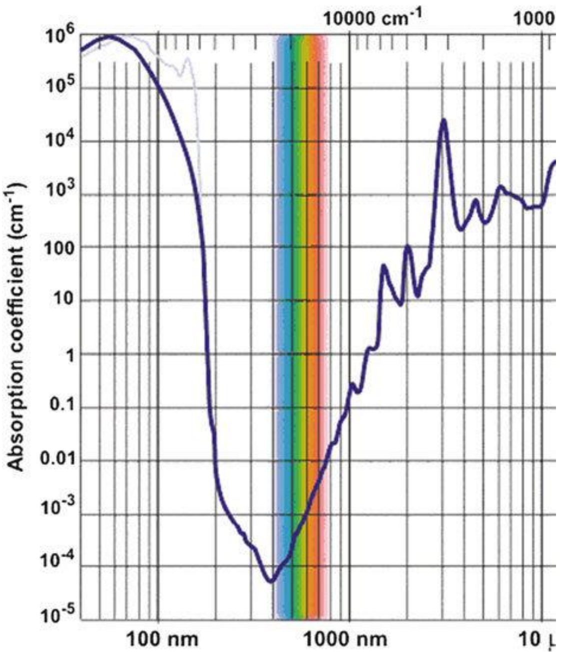

Fig. 7. The absorption spectrum of water
Source: M. Chaplin, Water Structure and Science https://water.lsbu.ac.uk/water/water_vibrational_spectrum.html Jagger and Sands, in 1999, showed that the octopus has chromatic aberration in their view [15]

Fig. 8 presents an image illustrating the chromatic aberration characteristic of octopus vision, distinguishing it from other aquatic organisms.

3.2 Microbiological Studies

Microorganisms isolated from the external surface of the examined octopus and their quantities are presented in Table 2.

Fig. 8. Picture of the vision of *O. vulgaris*

Microorganisms isolated from the external surface of the examined octopus and their quantities are presented in Table 2.

Using the Student's t-test for the study of triple-made tests, the total number of microorganisms yielded a p-value of <0.05 for both the head and tentacles.

The summarized data in the table indicate that more types and quantities of microorganisms are on the head of the studied octopus than on the tentacles. Staphylococci predominated, represented by three different species. Among the Gram-negative bacteria, *E. Coli was* detected only on the head but not on the tentacles. *Klebsiella pneumoniae ssp. ozaenae* and *Aeromonas hydrophilia ssp*. hydrophilia were also found. Results from serological typing of the isolated *E. coli* indicated that it belonged to group III, encompassing serogroups O6K, O20K17, O124K72, O125K70, and O126K71. In addition to the bacteria in the table, individual colonies of *Enterococcus caseiflavus* and *Candida lusitaniae* have been isolated from the surface of the octopus.

The results indicated that the head and tentacles of the octopus exhibit specific characteristics for the predominant growth of certain microorganisms. Microorganisms isolated show significant adaptability to pH, and no results that demonstrate a pH-dependent relationship with the pathogen counts were obtained.

Table 3 presents the results of the antibiotic susceptibility testing and illustrates the *in vitro* sensitivity of most isolated bacteria from different groupsto antimicrobial agents.

Table 2. Types and quantities of microorganisms isolated from the external surface of the examined octopus

Microorganism species	Number of Microorganisms – CFU cm^{-2} surface	
	Head	Tentacles
Escherichia coli	2.67+0.47	1.67+0.47
Klebsiella pneumoniae ssp. ozaenae	4.00+1.63	-
Aeromonas hydrophilia ssp. hydrophilia	9.67+2.05	-
Staphylococcus simulans	107.67+10.21	67.33 + 6.02
Staphylococcus piscifermentans	14.33+1.69	37.33 + 1.25
Staphylococcus hyicus	3.67+1.25	14.67 + 3.39
Total (all microrganisms)	713.34+34.85	673.34 + 33.44

Table 3. Sensitivity of isolated bacteria to antimicrobial agents *in vitro*

Antimicrobial agent	Content of disk	Inhibitory zones in mm and sensitivity of the strains				
		S.simulans	*S.piscifer-mentans*	*E. casei-flavus*	*E. coli*	*K.pneu-moniae*
Chloramphemicol	30 µg	25 (S)	24 (S)	20 (S)	25 (S)	25 (S)
Tetracycline	30 µg	29 (S)	19 (S)	25 (S)	23 (S)	21 (S)
Clindamycin	10 µg	14 (R)	17 (I)	12 (R)	14 (R)	11 (R)
Penicillin	10 u	23 (I)	32 (S)	16 (R)	11 (R)	6 (R)
Oxacillin	1 µg	9 (R)	15 (R)	11 (R)	9 (R)	10 (R)
Ampicillin	30µg	18(S)	24 (S)	13 (I)	6 (R)	8 (R)
Amoxycillin	25 µg	22 (S)	30 (S)	12 (R)	6 (R)	12 (R)
Cefuroxime	30 µg	15 (I)	16 (I)	18 (S)	11 (R)	14 (R)
Cefotaxime	30 µg	10 (R)	11 (R)	10 (R)	20 (I)	15 (I)
Novobiocin	30 µg	13 (I)	14 (I)	10 (R)	9 (R)	22 (S)
Streptomycin	10 µg	16 (S)	16 (S)	20 (S)	15 (S)	17 (S)
Gentamicin	10 µg	17 (S)	19 (S)	17 (S)	16 (S)	17 (S)
Amikacin	30 µg	21 (S)	19 (S)	19 (S)	18 (S)	20 (S)
Kanamycin	5 µg	9 (R)	12 (R)	10 (R)	9 (R)	6 (R)
Enrofloxacin	5 µg	26 (S)	25 (S)	21 (S)	29 (S)	25 (S)
Sulfamethoxazole+ Trimethoprim	23,75/ 1,25 µg	12 (R)	11 (R)	9 (R)	18 (S)	21 (S)

S (sensitive); I (intermediate); R (resistant)

The presented data shows that the tested microorganisms exhibited significant similarity in their *in vitro* sensitivity to the applied antimicrobial agents from different groups. However, the investigated Gram-negative species demonstrated higher resistance to most of the tested antibiotics. All isolated strains were susceptible to broad-spectrum agents such as amphenicols, tetracyclines, aminoglycoside-aminocyclitol compounds (excluding kanamycin), and quinolones. Staphylococci were sensitive to some of the penicillins (Ampicillin, Amoxycillin). Nonetheless, lincosamides (clindamycin) provedineffective *in vitro* against the studied bacteria. Potentiated sulfonamides exhibited high activity against the investigated Gram-negative bacteria but not against the Gram-positive ones.

4. DISCUSSION

One of the intriguing questions in contemporary science is related to the adaptability of Earth organisms to extraterrestrial conditions. It is logical to assume that simpler organisms might adapt more to harsh external conditions. The results of our spectral analyses unequivocally show that the octopus has a different spectrum compared to that of jellyfish and seawater in the near and mid-infrared range. Interestingly, this spectrum is characteristic of many meteorites from Mars. Nearly all the observed peaks in octopuses are analogous to those found in Martian meteorites, whereas they are not observed in seawater and jellyfish.

The structure of octopuses with spectral peaks at 896, 933, and 1080 cm^{-1} is similar to that found in space [16] and on Mars [17]. The peak at 896 cm^{-1} is characteristic of the C−C stretch, indicating traces of chemical processes in living matter with calcium oxalates [18]. As detected by infrared spectroscopy, Phyllosilicates exhibit a peak at 933 cm^{-1}, which is characteristic of cysteine [19]. A spectral peak at 1117 cm^{-1} has been reported for Martian meteorites [20]. Do Nascimento-Dias et al. emphasize the presence of organic compounds in meteorites of Martian origin [17]. Regarding the peak at 2927 cm^{-1} observed in octopuses, there is an analogy with their data in the range of 2920 to 2850 cm^{-1}, interpreted as organic components corresponding to aliphatic hydrocarbons. In the study of Martian meteorites from the SNC group using synchrotron FTIR spectroscopy with ~1 µm spatial resolution in the mid-infrared range (4000-850 cm^{-1}), Yesiltas (2018) identified the presence of aliphatic-type organic substances, as well as OH (at 3000 cm^{-1}) [21]. The authors reported small but pronounced peaks at 2961, 2924, and 2852 cm^{-1}, which are characteristic of hydrocarbon chains. The reactions of calcium carbonates and calcium hydrogen carbonates were studied in hot water with polar molecules in water and solid surfaces [22, 23]. We also observed a distinctive rise at 2961 cm^{-1} in our spectral analyses of octopuses. The peak at 3414 cm^{-1}, detected in octopuses, is characteristic of weak carbon bonds in water [24].

Osmotic pressure and the influx of seawater are crucial for reproducing octopuses as they provide the primary mechanism for the spermatophore reaction. The external envelope of the spermatophore acts as a semi-permeable membrane that allows seawater but not macromolecules to pass through [25].

It has been proven that Mars has liquid water, not only in the form of ice but also in a liquid state. Approximately 540 million years ago, during the Cambrian explosion on Mars, there was liquid water with salts [26]. Liquid water has been abundant on Mars over the last several million years [26]. This implies the possibility of biological forms of life at that time. Ling &Wang (2015) highlight that the changing conditions on Mars may have been similar to those in Earth's Antarctica [27]. A peak at 1454 cm^{-1} has been identified as indicative of the presence of water on early Mars [28]. Their spectral analyses also show the presence of cyanobacteria in artifacts of Martian origin.

In connection with these findings, we hypothesize that if Mars (or another similar planet) once hosted life and multicellular organisms, the following scenario may have occurred. A decrease in the amount of water leads to the concentration of salts in it, a drop in temperature, and freezing of the concentrated water basins and the living organisms in them. These processes may not have perished but entered hibernation due to cryoprotection from the focused water components); eventually, the loss of the atmosphere might have accompanied this, and a kind of natural lyophilization occurred in the created vacuum. Living organisms could have survived indefinitely in a frozen state at very low temperatures. Frozen eggs could have been stored in icy comets, and inside the comets, there would be no damage from cosmic radiation. Carried by a comet (composed of icy pieces), they could be transported through space to other planets. The salts and components of saltwater, especially if they were in higher concentrations on the originating planet, could perform a similar function. In a frozen state, the organisms could be transported (after being carried by a recently passing comet) and subsequently "released" on Earth when the comet passed near or entered Earth's atmosphere. Friction with the atmosphere would lead to thawing. If it happened over a water basin, the living organisms would enter a new aquatic environment and survive after adapting to the new conditions.

Hydration (entering a water environment) would be necessary to restore these organisms' life functions. The octopus differs from other Earth organisms in several biological aspects. 33,000 protein molecules have been identified in octopuses, many more than in humans, where they number 24,000 [29]. Octopuses can uniquely alter their RNA [30]. Furthermore, they are characterized by a high calcium content in their biochemical composition (Villanueva & Bustamante 2006), while seawater has the highest sodium content. This suggests that over time, octopuses have adapted to Earth's seawater. Calcium carbonate has also been found in Martian meteorites. According to Ignatov et al. $CaCO_3$reacts dynamically with the border mediums – air, water, and land [23]. This is illustrated by the Raman shift between 1080-1090 cm^{-1} in NWA 6963 and ALH84001, termed the “internal mode" as it arises from vibrations between C and O in carbonate (CO_3) and is indicative of the presence of calcite ($CaCO_3$) [17]. Ksanfomality et al. (2019) have presented evidence about specific types of life on Venus. "Venus-9-14" spacecraft have captured objects from the flora and fauna of the planet Venus, represented by the "TV-image" method [31]. The objects could show the existence of life on Venus under physical conditions radically different from those on Earth.These results suggest that life may be able to spread over relatively short cosmic distances, even within the solar system and the Milky Way.

Another interesting distinction between octopuses and Earth organisms is that octopuses exhibit chromatic aberration in their vision, meaning they see a spectrum different from that of other marine organisms [32]. They evolutionarily perceive the blue color, determined by the water absorption spectrum. The diversity of eye designs and light-sensing mechanisms evolved in the ocean is more significant than on land due to a more excellent range of light habitats [8]. Before the appearance of terrestrial life, aquatic organisms evolved the ability to see in a small region of the electromagnetic spectrum, 300–700 nm. This range is determined by two physical limitations that govern underwater vision: the similarities in the refractive index of animal tissue and water (as opposed to air) and the rapid attenuation of light over distance underwater. The octopus is believed to be color blind. While most animals in the ocean are colorblind monochromats, due to the volume of the habitat, color vision systems exist up to the twelve-channel retina of the stomatopod. However, many species, including the dominant fish and crustaceans, are dichromats even in surface waters. Smaller fish generally have UV spectral sensitivity, unlike larger predators. The ocean also has various trichromats (with three spectral sensitivities). In contrast, almost all birds possess one of two, not so distinct, tetrachromicities: that is, four spectral sensitivities spread relatively evenly across the spectrum, sensitive to ultraviolet/violet, blue, green, yellow, and red. Shallow-dwelling freshwater fish, such as goldfish or guppies, also tend toward tetrachromacy, and this is an optimal color vision system working in the 300–700 nm spectrum [8]. Most crustaceans, including deep-dwelling stomatopods, are monochromats or dichromats in marine habitats. Interestingly, many larger marine animals, including sharks and cetaceans, are also prone to bicolor. Dichromatic color vision first evolved in the ocean to eliminate flicker. Monochromats, which would suffer from the flicker, are adapted to live at great depth or at night when flicker is absent [8].

The results we obtained are unsurprising, as staphylococci are halophilic bacteria, and it is normal for them to be among the prominent representatives of the normal microflora on the surface of marine organisms. However, *S. simulans* is conditionally pathogenic for animals and humans. The same applies to most isolated Gram-negative bacteria - *E. coli* and *K. pneumoniae* [33]. Their presence on the surface of the studied octopus may indicate marine water pollution and the likelihood of the presence of other pathogenic species. The *E. coli* isolates belonged to group III (O6K, O20K17, O124K72, O125K70, and O126K71) that includes pathogenic to humans diarrheagenic serotypes, specifically enterotoxigenic *E. coli-ETEC* (O6, O20), enteroaggregative *E. coli* (EAEC) (O124), and enteropathogenic *E. coli-EPEC* (O125, O126) [34-36].

Aeromonas hydrophilia is pathogenic to marine life, including octopuses. The *Klebsiella* species we isolated as part of the normal microflora on the head of an octopus are conditionally pathogenic. They are characterized by the formation of exopolysaccharides that help them to adapt and survive in different ecological niches. They also form biofilm capsules and are an essential virulence factor [5]. Not only *A. hydrophila* but also *K. pneumoniae* and *E. coli* are pathogenic to octopuses [37], and their presence on the skin of the specimen we examined indicates that in case of injuries and other adverse conditions, they would show their pathogenic potential. *E. casseliflavus* isolated by us is also pathogenic in humans, especially in immunocompromised and chronically ill patients, and can cause bacteremia, endophthalmitis, endocarditis, meningitis, peritonitis, and pyothorax [38].*Candida lusitaniae* is a rarely reported opportunistic pathogen that can cause fatal infections [39, 40]. In our opinion, the greater mobility of the tentacles, accordingly, their friction in the water environment is essential for the mechanical removal of part of the microorganisms on them and, respectively, the poorer in terms of types and amounts of microflora.

Our results are consistent with those of Farto et al., who reported that species of the genera *Vibrio, Pseudomonas, Aeromonas,* *Staphylococcus,* or *Streptococcus* are significant representatives of the normal microflora of healthy octopuses in their natural environment. Some of them are found in skin ulcers of octopuses; as they multiply, they can penetrate the body and cause an infection with a fatal outcome. Skin lesions often contain different types of bacteria at the same time. Our results are also consistent with those of Gullian-Klanian et al., who pointed out that the microbiological burden of *O. vulgaris* is less than that of other species, such as the red octopus *O. maya* [41]. The authors explain this with the greater depth at which *O. vulgaris* is caught.

Due to the development of some microorganisms from its normal microflora, after 72 hours of refrigerated storage at 4°C, the octopus loses quality, decreasing from category A to B, with the microbial count reaching log 4.7 CFU/g. According to Gibelloet al. [1], *Enterococcus* spp. can be misidentified as *L. garvieae* of the family *Streptococcaceae*.

De la Cruz-Leyva et al. have identified the bacterial flora associated with commercial *O. maya* caught in the Yucatan Peninsula [5]. They have found significant microbial diversity including *Carnobacterium* sp*., Lactococcus piscium, Lactococcus* sp*., Vagococcus* sp., *Psychrobacter* sp*., Psychrobacter urativorans, Pseudomonas* sp*., Pseudoalteromonas* sp*., Shewanella* sp., *Shewanella baltica, Klebsiella oxytoca, Vibrio aestuarianus, Photobacterium* sp*., Flavobacterium* sp., *F. antarcticum, Bizionia*sp*., Bacillus* sp*., C. divergens, Cetobacterium somerae, Psychrilyobacter atlanticus, Salinimicrobium* sp., as well as still unclassified species of the family *Flavobacteriaceae*. Obviously, the different latitude and climatic conditions are important for the differences in the microflora of this octopus species compared to what we found. Environmental factors such as water temperature and geographic location influence microbial diversity in octopuses. The sex of the animal can also have an effect, with the predominant species in female specimens being from the families*Vibrionaceae* and *Streptococcaceae*, while in males - mainly from *Vibrionaceae* [10, 42]. The octopus has mainly benthic habits, which means it is in close contact with the marine sediments. Bacteria from the groups *Firmicutes, Actinobacteria, Proteobacteria,* and *Bacteroidetes* predominate in these sediments, mainly from the genera *Pseudoalteromonas, Bacillus,* and *Photobacterium* [43]. The bacterial flora we identified is part of the natural microbial diversity of *O. vulgaris* caught in the Aegean Sea off the coast of Chalkida.

Aquatic products risk rapid microbial growth due to their chemical composition (high water, protein, and lipid content), aquatic habitat, and post-harvest handling [44]. Microorganisms can have positive effects, with some being used as probiotic agents, quality indicators, and flavor enhancers in seafood. Others hurt product quality and can also cause food poisoning, especially when consumed without heat treatment [5]. The carriage of beneficial microorganisms by aquatic inhabitants was also reported by Dhong et al., who have isolated from salted small octopus the species *Pediococcus pentosaceus* SC11 (GRC-SC11), which is a probiotic with high immunostimulatory activity [45]. This species could be a potential therapeutic agent for immunocompromised patients.

The presence of various types of bacteria on and in the body of aquatic inhabitants, including those pathogenic to humans, is the result of direct contact with a polluted aquatic environment and ingestion of bacteria from sediments or contaminated food. Therefore, the microorganisms found in aquatic inhabitants, including octopuses, reflect the condition and safety of the aquatic environment. Some pathogenic bacteria can cause severe diseases in fish; for example, *Yersinia ruckeri* is the causative agent of enteric red mouth disease in rainbow trout [11]. The primary pathogens for fish and other aquatic animals fall into two groups, some originating from natural freshwater habitats and others associated with water pollution. These are mainly *Vibrio spp., Yersinia spp., Salmonella enterica, Listeria monocytogenes, and Clostridium botulinum*, referring to both groups. The danger is significant due to the property of these bacteria to survive in the environment [11].Furthermore, they can enter the food chain, and seafood processing can lead to cross-contamination of premises, equipment, and finished products and thus spread. Adherence to the rules of good hygiene practice is a measure to avoid such contamination and ensure marine products' safety [11].

Fichiet al. [46] have investigated pathogens that caused skin lesions in Italy's 9 naturally deceased octopuses (*O. vulgaris*) [46]. They have isolated *Vibrio algynolitycus* and *Vibrio parahaemolyticus* (from cultured octopuses), *Lactococcus garviae,* and *Photobacterium swingsii*. Farto et al. [47] have studied the main bacterial pathogens associated with larval stages of cultivated *O. vulgaris*, juveniles, and adult ones [47]. They also reported that species of the genus *Vibrio* were the main bacteria associated with skin lesions in adults. Still, some were isolated from sterile organs or fluids, including *Pseudomonas, Aeromonas, Bacillus,* and *Micrococcus*. *Photobacterium swingsii* and *Lactococcus garvieae* have been found in a retrobulbar lesion in an octopus. These authors draw attention to the fact that the microflora of marine organisms is influenced by temporal changes in the environment, as well as by latitude, temperature, and water salinity. However, some bacteria are specifically and permanently associated with particular marine animals and are not found in seawater or on other animals, living in symbiosis with the host and providing it with some degree of protection against pathogens.

Solomon et al. [47] highlighted the dangers of seafood transmission of infections caused by *E. coli* and species of the genera *Vibrio, Salmonella, Staphylococcus,* and *Bacillus* [47]. Fish and seafood products must not carry *Salmonella enterica*, a total number of microorganisms above 10^5 CFU/g, and *Staphylococcus aureus* above 10^2 CFU/g. Some of these bacteria acquire resistance to antibiotics, primarily by receiving extra-chromosomal DNA with genes encoding resistance. The infections they cause cannot be treated with conventional antibiotics, which is a worrying trend. Seafood can be contaminated with pathogens of fecal origin, mainly in the pollution of water bodies.

The resistance we found in most of the tested antimicrobials indicates the widespread distribution of polyresistant strains, even among bacteria, which is representative of the normal microflora of marine inhabitants such as octopuses. This confirms the alarming trend of the spread of polyresistant microorganisms even in large water basins, as reported by other authors. Our results are consistent with those of Solomon et al., who reported higher antibiotic susceptibility of Gram-positive bacteria than Gram-negative species [47]. The highest resistance (100%) of the microorganisms isolated from them was registered against amoxicillin, augmentin, chloramphenicol, gentamicin, erythromycin, tetracycline, streptomycin, and cotrimoxazole, and the lowest - to ofloxacin, ciprofloxacin, and ceftriazone. Alarmingly, the species isolated by these researchers from the genera *Salmonella, Klebsiella, Staphylococcus, Bacillus,* and *Proteus* showed 100% resistance to all antibiotics tested [47]. Gibelloet al. reported susceptibility of their isolated *L. garvieae* to lincomycin, oxytetracycline, and macrolide antibiotics and resistance to several antimicrobial agents such as clindamycin, erythromycin, streptomycin, tetracycline, oxytetracycline, florfenicol, and some quinolones [1]. The differences in the antibiotic sensitivity of the bacteria isolated by us and by other authors are related to the wide use of antibiotics from different groups in the different geographical regions studied and areindeed related to anthropogenic pollution of water bodies with organic waste. In the future, improving the purity of seawater will reduce pathogenic microorganisms. This will lead to preserving a more significant number of octopuses as a unique natural species.

5. CONCLUSIONS

The spectral analyses of FTIR on octopuses, jellyfish, and seawater from Chalkida, Evia Island, Greece, reveal the presence of two peaks in octopuses at 896 and 933 cm^{-1}, also identified in Martian meteorites. These results provide us with grounds to speculate that octopuses may have originated from Mars or another planet with similar characteristics. However, the spectral characteristics of jellyfish and seawater do not correspond to the spectral features found in Martian meteorites.

The octopuses we studied were mainly carriers of staphylococci (*S. simulans, S. piscifermentans,* and *S. hyicus*), halophilic bacteria. Their surface microflora also included microorganisms conditionally pathogenic for animals and humans, such as *S. simulans, E. coli, K. pneumoniae, Enterococcus caseiflavus, Candida lusitaniae*, as well as *Aeromonas hydrophilia*, which is pathogenic for marine inhabitants, including octopuses. The presence of these species on the surface of the examined octopuses, especially of *E. coli*, could indicate fecal pollution of the seawater and the possible availability of other pathogenic species.

All bacteria we isolated showed resistance *in vitro* to clindamycin, penicillin, oxacillin, kanamycin, and almost all - to cephalosporins and novobiocin. They were sensitive to broad-spectrum chloramphenicol, tetracycline, gentamicin, amikacin, enrofloxacin, and streptomycin. The potentized sulfonamides showed high activity against the examined Gram-negative bacteria but not against Gram-positive ones. Ampicillin and amoxicillin were effective *in vitro* only against isolated staphylococci. The present results show the availability of polyresistant bacterial strains even among bacteria representing the normal microflora of octopuses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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