Applications of Microwave Energy in Medicine

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Abstract: Microwaves are a highly utilized electromagnetic wave, used across a range of industries including food processing, communications, in the development of novel medical treatments and biosensor diagnostics. Microwaves have known thermal interactions and theorized non-thermal interactions with living matter; however, there is significant debate as to the mechanisms of action behind these interactions and the potential benefits and limitations of their use. This review summarizes the current knowledge surrounding the implementation of microwave technologies within the medical industry.

Keywords: microwaves, medicine, bacteria

1. Introduction

Microwaves are a section of the electromagnetic (EM) spectrum (Figure 1; [1]): this spectrum ranges from radio waves to gamma rays. The EM spectrum can be expressed as frequency, which is measured in Hertz, wavelength and energy. Shorter waves with a higher value of energy such as ultraviolet are classed as ionizing as they generate sufficient energy to produce ions at a molecular level, causing damage to DNA and proteins. Whilst longer waves such as visible light are classified as non-ionizing, these can still cause thermal damage, however, this damage is not caused through ions. Microwaves are a type of electromagnetic radiation with free-space wavelengths ranging from 1 meter to 1 millimeter, with the frequency ranging between 300 MHz and 300 GHz, respectively [2]. The most common microwave frequency used is centered at approximately 2.45 GHz, which lies within the Industrial Scientific and Medical (ISM) radio band and is reserved for such purposes [3]. In recent years, microwave energy has been successfully exploited within medicine to treat diseases such as cancer and microbial infections via ablation therapy. However, there is now increased interest in using a range of microwave frequencies other than 2.45GHz in treatment of diseases; however, there is still limited understanding of the mechanisms of action of microwaves which induce biological changes in organisms. In this review, we focus primarily on microwave interactions at a cellular level with bacteria as model organisms. Herein we examine current literature regarding the functionality, current and prospective uses of microwave energy across a range of frequencies to demonstrate state of the art microwave advances in the medical industry (Figure 2) [4,5].

2. Electromagnetic Fields

An electromagnetic field consists of both a magnetic and an electric field produced by positively or negatively charged particles (Figure 3;[2]). An electric field is generated when particles gain a charge, either positive or negative via the transfer of electrons. If the electrically charged particles start to move, they produce an electric current; this current produces a magnetic field around the electric current. The electric field does not have to be moving in order to produce a magnetic field, if the charge of the electric field is fluctuating then a fluctuating magnetic field will be induced. Due to their
Both magnetic and electric fields are bound by laws of attraction which state that opposite charges always attract while ‘like’ charges repel. The strength of the attraction or repulsion is negatively proportional to the distance of the charges. One of the best examples of these laws is the chemical bonding between atoms via charged electrons and protons, these interactions can be described mathematically with Coulomb’s law [7]. Magnetic fields are produced by the presence of two charges that create field lines, where these lines intersect is described as poles, an example of this is the Earth’s North and South pole, such magnetic charges can only exist as dipoles, not monopoles. Mathematical equations such as Maxwell’s equations prove the model for electromagnetism, furthermore these equations describe how fluctuating electric and magnetic fields (Figure 3) travel at a constant speed [8]. Electromagnetic fields can act as waves and particles simultaneously; the waves travel outwards from their source and can move through a medium or through a vacuum. In a vacuum, the wave travels at the speed of light, similarly the air in our atmosphere is thin enough not to affect the propagation of the wave, however when travelling through media the refractory index of the media will affect the waves movement. For media such as water there are other factors that alter the propagation; the high permittivity and electrical conductivity of water greatly increases the angle of refraction [9, 10]. Another factor to consider is the microwaves ability to interact with polar molecules; water molecules are polar and so as the microwave passes through the water as a medium it also interacts with it. Polar molecules rotate when exposed to microwaves as they attempt to align with the waves’ fluctuating charges; the rotation produces heat and is the basis of microwave heating [11].

3. Thermal Interactions with Bacteria

Microwaves are commonly used to heat food whilst reduce the microorganisms found within. Microwave heating reduces the number of microorganisms within food via direct thermal killing of cellular targets that render the bacteria either dead or inactive and therefore unable to replicate [12]. The microwave heating process relies on the interaction between polar molecules and the microwaves; in a 2.45GHz microwave oven, the microwaves’ frequency is strong enough to cause water molecules in food to rotate at a speed which generates heat to cook food safely (Figure 4) [13]. Bacterial walls, capsules and the media in which the bacteria are cultured contain polar molecules that will rotate and produce heat when exposed to microwaves [14, 15]. Direct thermal killing results in the death of the bacterial cell by an increase in temperature which in turn severely damage the peptidoglycan wall. In Staphylococcus aureus the cell loses D-alanine from the teichoic acids, this results in the cell’s inability to perform certain metabolic processes. Proteins are directly damaged by heating as the bonds holding them together are destroyed, this can damage enzymes and structural proteins that result in a loss of functionality [16, 17, 18]. Heat can also affect the integrity of many cellular aspects causing the cell to become inactive, in Gram negative bacteria the outer membrane is damaged by heat and becomes sensitive to lysozyme and hydrophobic antibiotics [19]. Due to the dependency microwave heating has with polar molecules, dried samples are not affected due to the lack of polar molecules, while those in the presence of water are able to reach lethal temperatures [20]. There is debate that the non-thermal effects could contribute to the mechanism of destruction, as at sub lethal temperatures enzyme activity is altered in S. aureus and in turn, could result in a change in bacterial functionality [21].

4. Non-thermal Interactions with Bacteria

Electroporation is one of the non-thermal effects believed to be caused by microwave irradiation. In this concept the microwaves at sub-lethal temperatures induce the formation of pores in a cellular membrane due to their interaction with polar molecules. Although this is yet to be fully understood, current theories suggest that the polar molecules in the cell membrane rotate and create reversible...
pores; once the microwave is removed the pores close and returns to the original structure [22, 23, 24, 25]. These pores allow the cellular contents to leak outside, including substances such as DNA that are not normally able to cross the cell membrane. These released components from the cell are fully intact at sub lethal temperatures and once purified can be used for further research and pure DNA separated from other cellular content can be used for identification [26]. Cells have shown to initially shrink after non-lethal microwave exposure, however, once the pores close, within 30 seconds the cell has been observed to return to its original size [21, 26]. Utilising electroporation in the development of medical treatments and diagnostics is desirable due to its speed and low cost. Many researchers have found that electroporation is a viable tool for identification, DNA extraction and as a delivery system for molecules into cells, some of these developments and methods are discussed below.

5. Healthcare Developments

Microwaves have a wide variety of uses within and outside of healthcare. In the 1950’s microwaves were developed for communication and later for navigation with the use of relay links and satellites utilizing their properties as electromagnetic waves [27,28]. However it is the interactions with polar molecules in substances that has led to the development of many uses within the healthcare setting. The thermal interactions have been adapted for sterilization, sample preparation and ablation of cancerous cells, the rapid heating and thermal killing induced by the microwaves interactions with polar molecules makes this viable and effective. While the non-thermal interactions with polar molecules are being exploited for microwave imaging and extraction of intracellular components for rapid diagnostics, the permittivity of the wave and mechanism of electroporation make these uses practical although not yet perfected [29, 30].

Sterilisation via microwave exposure has been developed through microwave heating and a series of treatments. Microwaves between 225MHz to 100GHz are primarily suited for sterilisation: the primary microwave used for heat sterilization of food in this range is 2.45GHz [31]. 2.45GHz microwaves have also proven to be able to sterilise glass and plastics in as little as 180 seconds, this too requires the presence of water within the microwave to act as a heat sink and interact with the electromagnetic waves. This method of sterilisation can be used on both laboratory and medical equipment in place of an autoclave [32, 33].

Another use that makes use of thermal interactions is Microwave Metal Sample preparation through microwave digestion. Microwave digestion is a technique to dissolve heavy metal in the presence of organic matter. This process exposes samples to strong acids and then raises the temperature using focused microwaves. This method can be used for environmental samples to measure for contaminants that could affect human health, such as lead. In order for samples of soil to be analysed they must be transformed into liquid samples through microwave digestion. The samples can then be analysed via Inductively coupled plasma mass spectrometry (ICP-MS) for trace metals or flame atomic absorption spectroscopy (FAAS) for major elements [34]. Microwave digestion and the subsequent spectrophotometry can be used to analyze trace metals in human tissue such as hair, nails and gallstones. Gallstones have trace amounts of metal that are associated with bilirubinate and black pigmented gallstones, thus by determining the amounts and variety of metals within gallstones their origin of these metals may be determined [35,36].

Ablation therapy is a state of the art treatment and destructive tool for abnormal tissues via heating by radiowaves or microwaves [37]. Microwave ablation is a method of thermal tumour ablation where tumours are heated in order to damage the cells structure and proteins. As tumours have a higher water content than healthy tissues, the microwaves induce rapid heating via interacting with the polar water molecules within the tumour cells. Due to the microwaves ability to propagate through media, it can pass through and heat various tissues making it an applicable thermal ablation therapy for a variety of tissues [4, 5, 38, 39]. Microwave ablation therapy for hepatocellular carcinoma
is being viewed as a potential first line treatment for tumours on the liver surface, in which the
tumour is exposed to a 2.45 GHz microwave with a wattage of 80-100 (Figure 5) [5, 40]. Microwave
ablation therapy has not only been successful within the liver, clinical studies have also shown
complete ablation of tumours within the kidneys, lungs and bone, the majority of studies have
resulted in no recurrent tumours [41]. Despite the success of microwave ablation the use of this
treatment is not as widely practiced as expected, this is partly due to the expertise and equipment
needed to perform the therapy, alongside competing ablation therapies, such as radiofrequency
ablation therapy [42, 43, 44]. Radiofrequency ablation yields similar successful tumour ablation
results, however there are advantages to microwave ablation, microwaves produce higher
temperatures with shorter ablation times and a smaller heat sink effect [45, 46]. Ablation therapy is a
key development in the treatment of tumours, allowing the destruction of them without the need for
surgery, the increased usage of both ablation techniques could reduce the cost and time needed to
treat small tumours [47, 48].

Microwaves can play a role in both the detection and treatment of breast cancer. Microwave imaging
has been researched as an alternative to X-rays and ultrasound screening which have a variety of
limitation [5, 49]. Microwave imaging is an appropriate alternative as it is low cost, harmless and
potentially easier to preform compared to current methods with high sensitivity. Imaging techniques
rely on the knowledge of the permittivity and conductivity of malignant, benign and healthy breast
tissue. Due to the higher water content in cancer cells the dielectric properties of the tissue differs
when exposed to microwaves [50]. Despite the nearly 40 years of research into microwave breast
imaging there are still many limitations that prevent a commercially available device which includes
inappropriate algorithms and sensors; however, with wider clinical trials a viable microwave
imaging system may be feasible in the near future [39, 40]. Microwave ablation has also been trialed
for the treatment of breast cancer and so far has proved to be successful in thermal ablation [51,52].

Other than ablation and rapid heating, treatments for damaged and keratinized cells have been
researched and developed with controlled heating. The Swift® microwave is an approved state of
the art treatment for plantar warts caused by the human papilloma virus (HPV). Directly exposing
the wart to 8GHz microwaves at 8 Watts for 2 seconds causes the wave to interact with the keratinized
skin and result in controlled heating of the tissue. There is also the suggestion that microwaves
enhance the cross-presentation of dendritic cells that are key for the immune defense against HPV
[53, 54]. A similar method has been developed for the potential treatment of actinic keratosis (AK).
The Swift 8GHz® microwave is used to expose the ulceration to 4W for hyperkeratotic AK or 3 Watts
for nonhyperkeratotic AK for 3 seconds repeated in triplicate with a 20-second time gap between
pulses. This treatment has resulted in the clearance of actinic keratosis with brief pain and minimal
long term adverse side effects in 90% of applied sites [55]. Following this the use of microwaves to
treat benign cancerous and precancerous lesions caused by high-risk HPV has been investigated.
High-risk HPV results in an increased expression of 2 viral oncoproteins; E6 and E7. When in vitro
grown tumors were exposed to microwaves for 10 seconds both tumor cell death and a reduction in
E6 and E7 in the treated zone and transition zone were observed. This reduction suggests that
microwave interactions can reverse the cancerous phenotype caused by HPV; and that once an
effective and proven method is developed it could provide a less invasive treatment for HPV benign
tumors [56, 57].

State of the art microwave-based molecular diagnostics that incorporate the non-thermal effects of
microwaves are currently under development in order to tackle the delays and complexities of
current methods; these include microwave assisted metal enhanced fluorescence and nanotube
assisted microwave electroporation. Microwave assisted metal enhanced fluorescence (MAMEF) is a
rapid diagnostic method being developed to detect bacterial infections at point of care. MAMEF
combines the use of silver nanoparticles deposited on microscope slides which are impregnated with
anchored DNA sequences specific to the target sequence - such as a bacterial species [58, 59]. Low
power microwave heating kinetically accelerates the hybridisation of the target DNA and a
fluorescent DNA target (usually a conserved region of bacterial genomic DNA) is excited and detected by the process. This method is currently crude and not yet manufactured for wider laboratory use [60]. If commercialized appropriately and manufactured into an integrated device, MAMEF would be a useful point of care diagnostic due to its speed, specificity, low cost and simplicity [61].

Lyse-It is advertised as a rapid “single step” process that lyases cells and causes DNA/RNA fragmentation. It uses a glass slide with gold nanolayers deposited in a “bow-Tie” shape. Using a sticky silicone isolator (Sigma Aldrich), the sample is placed on the slide in the centre of the gold bow and microwaved in the centre of a conventional 2.45 GHz microwave oven for lysis. The theory is that the gold focuses the microwaves to release the fragmented DNA [62]. This method of lysing DNA is much quicker than the current diagnostic DNA extraction methods and could be incorporated into point of care diagnostics. However, crucially there are currently no benefits in sensitivity as the samples are contaminated by the microwaved gold, and currently due to the cost of the Lyse It Slides this method is not yet suitable to be used as a common DNA extraction method within diagnostic laboratories [63, 64].

MAMEF and Lyse-It are not the only devices exploiting the proposed non-thermal effects of microwaves as a method of DNA extraction for molecular diagnostics. Methods using micro centrifuge tubes as an alternative to the expensive Lyse It slides are being researched, however these processes are still somewhat time consuming due to the required centrifuge and wash procedures [65]. Development of other rapid diagnostic devices that utilize microwave-based DNA extraction and fragmentation as a first step to sample processing are in the early stages of development [66].

Microwaves are also being used in DNA extraction outside of a healthcare setting, in environmental samples such as sediment and soil; these ecosystems are hard to culture due to the diversity of organisms and growth conditions, therefore genetic typing is the primary form of identification. There are limitations with current enzymatic lysis methods as the high molecular weight genomic DNA required for further research needs to be relatively pure, whereas the DNA from environmental samples have a variety of contaminants. Through implementing a microwave based thermal shock lysis method in which environmental samples are exposed to 2.45GHz, 600-700W microwaves for 45 seconds, a relatively large amount of good quality DNA (20-23kb) can be extracted; in a sample size of 300 μL of activated sludge, up to 50 μg of DNA was extracted via microwave thermal shock compared to the 30 μg extracted via enzyme based protocols. After the lysis of the environmental samples, appropriate washing and amplification can be performed for identification of the 16S ribosomal gene. The same thermal shock method can be used to extract RNA, followed by an alternative suitable washing and amplification step can be used for further identification of the 16S rRNA gene. This method of lysis is not only cost effective and quicker at identifying environmental samples but could be developed and implemented to detect infectious microorganisms within stool samples in patients in a healthcare setting once these methods have been proven to be robust [67, 68].

Diagnostic and identification methods that do not rely on the extraction of intracellular components have also been researched. Nanotube assisted microwave electroporation (NAME) is a method that utilises electroporation, NAME however aims for visual identification rather than DNA extraction [69]. This technique can be applied to not only extract the contents of a bacterial cell, but to act as a transport system to deliver molecules such as biosensors into the cells (Figure 6). In this method, carbon nanotubes are used as an antenna for coupling microwave energy; this localizes the electromagnetic field that induces bacterial electroporation in the cellular wall to the areas around the nanotube. This enhanced electroporation to specific areas (caused by the nanotubes) allows for the delivery of intracellular probes that consist of double stranded nucleic acid targeting specific bacterial 16S rRNA and fluorophores, enabling the identification of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* for example at the single-cell level. An individualized probe was designed for each bacterial species. Bacterial samples of *E. coli*, *K. pneumoniae* and *P. aeruginosa*,
after appropriate washing steps and the addition of the nanotube solution, were exposed to a 2.45GHz microwave for 10 seconds, enabling electroporation and probe delivery. Once the probe was delivered into the bacterial cell via NAME the samples were mounted onto glass slides for observation under a fluorescent microscope; cells that were fluorescent were accurately identified. This delivery and identification method can be used directly on samples, and the whole process from the initial sample to microscopic identification can take as little as 30 minutes. NAME can identify pathogens such as those mentioned previously at a single cell level enabling accurate quantification via cell counts of fluorescent cells under the microscope, however further instrument development is required before this method can be clinically evaluated [70, 71].

When comparing the state of the art microwave based DNA extraction methods with current commercially available DNA extraction kits, the overall opinion is that microwave methods are more efficient, cost effective and simpler, so could be implemented without the need for specialised training [62, 72]. The quantity and quality of DNA extracted by both techniques appear to be similar for cultured samples, while microwave techniques on specific samples such as blood can have varying results. If techniques and instruments are appropriately developed, microwaves have significant potential to enhance rapidity and development of point of care diagnostic devices, for example to tackle global healthcare challenges such as antimicrobial resistance [73]. The future perspectives of microwave use throughout the medical industry are increasingly positive. Microwave ablation has been approved as an effective treatment for a range of cancers with promising results to be implemented for use. If development of methods and equipment continue then this could be a cost effective, minimally invasive method of treating a variety of sized and shaped tumours [39, 74]. Although positive the development of such techniques are not without challenges, controlling the direction and reflection of the wave is important in all treatments in order to not damage healthy tissue as well as the size of the delivery antennas [75]. Utilising microwaves for diagnosing breast cancer is in need of further research to refine the understanding of the dielectric properties of cancerous tissues and the required equipment. If these developments are made microwave imaging could be pain free, harmless and quicker than current screening methods such as mammography and X-ray [76].

6. Wider Impacts of Microwave Use

The impact of microwaves is widespread across medicine and has clear economic benefits; for example, the cost of cancer treatments could be reduced by thousands of dollars per person [77]. Another example is microwave sterilisation which, when compared to chemical sterilisation for medical equipment, has a variety of economic and environmental impacts. The production of microwaves responsible for sterilisation is expensive, but is a one-time cost other than routine equipment maintenance and the running cost of electricity needed for microwave sterilisation is also not small, however, environmental impact is limited in correlation to the production and power supply of the microwaves. Chemicals for sterilisation are cheaper to purchase in small quantities, however, this is no more expensive than microwave use on a larger scale. Moreover, the environmental impacts are high throughout the manufacturing process, use and disposal of the chemicals [78, 79].

The same economic and environmental effects of microwave use can be applied throughout all microwave developments. The cost of producing the microwave is high due to the resources, equipment and power required and therefore the environmental impact is high due to these resources being often non-renewable.

The disposal of microwaves is one of the greatest sources of environmental impacts in the process if disposed of incorrectly, however, due to the Waste Electrical and Electronic Equipment recycling (WEEE) directives a wide array of microwaves are recycled safely [80]. The environmental and economic impacts created from microwaves and point of care technology is always changing and
generally reducing due to advances in consumer electric drives; as new technology is developed the parties involved will be actively attempting to reduce their environmental footprint and act sustainably [81].

Table 1: Summary of Microwave Applications in Medicine

<table>
<thead>
<tr>
<th>Microwave Application</th>
<th>Energy</th>
<th>Method</th>
<th>Example(s)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilisation</td>
<td>Thermal Energy. 225MHz to 100GHz; 2.45GHz</td>
<td>Food, Glass, Plastics</td>
<td>[31-33]</td>
<td></td>
</tr>
<tr>
<td>Heavy Metal Digestion</td>
<td>Thermal Energy 2.45GHz</td>
<td>Metals, Gallstones</td>
<td>[34-36]</td>
<td></td>
</tr>
<tr>
<td>Ablation Therapy</td>
<td>Thermal Energy 8GHz, ~2.45GHz</td>
<td>Oncogenic Tumors, Keratinised cell, Plantar Warts (HPV)</td>
<td>[38-40],[42-44],[53-57]</td>
<td></td>
</tr>
<tr>
<td>Diagnostics</td>
<td>Non Thermal Energy Microwave Accelerated Metal Enhanced Fluorescence (MAMEF) 2.45 GHz</td>
<td>Bacterial pathogens</td>
<td>[58-62]</td>
<td></td>
</tr>
<tr>
<td>Lysis</td>
<td>Thermal Energy 2.45GHz</td>
<td>Bacterial Pathogens</td>
<td>[63,64]</td>
<td></td>
</tr>
<tr>
<td>Electroporation</td>
<td>Non Thermal Energy 2.45GHz</td>
<td>Bacteria at single cell level</td>
<td>[69]</td>
<td></td>
</tr>
</tbody>
</table>

7. Conclusion

Microwaves are a versatile electromagnetic wave with a variety of uses within and beyond medicine. Currently 2.45 GHz microwaves are primarily used for heating and sterilisation within the food industry and the thermal methods of this process are relatively well known. These microwaves have also been adapted for medical treatments, microwave ablation therapy is available for a variety of cancers and reduces both the cost and length of treatment. The 8GHz microwave frequency has recently been utilised for the treatment of viruses and reduces the need for antimicrobial drugs. There are many areas in which microwaves are in development within medicine both in diagnostics and treatments, however despite all these advancements in the uses of microwaves, the knowledge behind the mechanisms as well as the impact microwaves have are limited. There is no clear mechanism behind the non-lethal interactions between microwaves and bacteria due to the limited control over the temperature and direction of microwaves there are still many risks involved with current treatments, as microwaves interact with all polar molecules. The mechanism is not the only knowledge gap that needs to be filled, limited studies regarding the environmental and economic impacts of microwave use hinder the appropriate development of microwave technology. Despite these limitations, the diverse applicability to human healthcare will improve the equality and longevity of life for many, with a reduced burden on economy.
8. Figures

**Figure 1.** Electromagnetic spectrum. A depiction of the range of frequencies and wavelengths in the electromagnetic spectrum and the sub ranges, as the wavelength increases the energy of the wave decreases [1].

- Microwave Energy in Medicine
- 3. Thermal interactions with Bacteria: The known mechanisms of how microwaves interact with polar molecules to generate heat.
- 4. Non-thermal interactions with Bacteria: The proposed mechanisms of how microwaves interact with bacterial polar molecules at sub-lethal temperatures, electroporation.
- 5. Healthcare Developments:
  - Sterilisation through microwave thermal interactions.
  - Microwave ablation therapy, thermal killing of cancer cells.
  - Microwave therapy for HPV, heating of the infected cells to trigger an immune cascade.
  - Microwave based extraction methods for bacterial intracellular components believed to be achieved through non-thermal electroporation.
- 6. Impacts of Microwave use:
  - The economic and environmental impacts of microwave manufacturing and use and the impacts of rapid diagnostic development utilising microwaves.
Figure 2: Summary of Topics Covered within Microwave Energy in Medicine. Diagram depicts key themes described in this review.

![Diagram of Microwave Energy](image1)

Figure 3: Electromagnetic wave diagram. Showing the direction of the wave, the direction and oscillation of the electric field and the direction and oscillation of the magnetic field. Each runs perpendicular to another; direction travelling along the X axis, electric along the Y axis and magnetic along the Z axis [1].

![Diagram of Electromagnetic Wave](image2)

Figure 4: Water molecules rotation to align with an oscillating electric field. The electric field in a microwave oscillates between positive and negative polarity, the water molecules negative oxygen and positive hydrogen particles rotate to abide by the laws of attraction. This rotation generates thermal energy.

![Diagram of Water Molecules Rotation](image3)
Figure 5: Microwave ablation schematic. The microwave antenna is made up of a applicator shaft that has temperature monitoring in order to combat shaft heating. Shaft heating occurs due to reflection of the microwave and a cooling system is installed along the shaft to prevent burning. The antenna is most often needle shaped but can have a variety of designs including monopole, dipole and slotted antennas. The antenna design determines the tissue heating pattern and therefore impacts the ablation zone size and shape [38, 39].

Figure 6: Nanotube assisted microwave electroporation (NAME) for single cell pathogen identification. Multiwall carbon nanotubes induce localized electroporation for the delivery of multicolour double stranded molecular probes for multiplex 16S rRNA detection [70]

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