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Original Research

Investigation of Bacterial Presence in Cerebrospinal Fluid by Bioimpedance Technique

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ABSTRACT

Objective: This study aimed to determine the presence of bacteria and their colony numbers in cerebrospinal fluid (CSF) by bioimpedance measurements at 50 kHz phase angle (PA) and 5 kHz impedance values.

Methods: We evaluated the performance of the 18 Gauge probe for detecting several types of bacteria in CSF *ex-vivo*. We equally monitored the electrical differentiation between colony numbers. A quantity of 200 µl sterile CSF was used as the standard in each experiment and was inoculated with 1, 2, 5, and 10 colonies of Coagulase-negative staphylococci, *Streptococcus pneumoniae* and *Acinetobacter baumannii*, separately.

Results: PA and impedance values of CSF samples were compared with each other concerning different colony numbers. It was observed that, varying numbers of colony strains of *Acinetobacter baumannii*, Coagulase-negative staphylococci, and *Streptococcus pneumoniae* could be differentiated from sterile CSF using PA and impedance values. Only one colony of the *A. baumannii* strain could not be distinguished from sterile CSF due to its thin cell wall composition.

Conclusion: The bioimpedance probe was time-saving and could detect the presence of bacteria in CSF samples correctly. Moreover, the probe can be used in the rapid detection of bacteria in CSF during real-time examinations.

Keywords: Bacteria, bioimpedance probe, cerebrospinal fluid

INTRODUCTION

Cerebrospinal fluid (CSF) is a clear, plasma-like fluid that bathes the central nervous system (CNS). CNS infections are essential and challenging perspectives of clinical neurology. Immediate and accurate examination enables clinicians to introduce effective therapies; however, in conditions without the correct diagnosis, the patients may suffer from serious neurological deficits and sometimes even death. The CSF reflects the pathology of CNS and helps in early diagnosis and therapy (1). CSF culture is the gold standard method for the detection of bacteria. In CNS infections, the CSF culture results are positive in 70%–85% of the cases who have not received prior antimicrobial treatment. The identification of the causative agents may take up to 48 hours.

Impedance (Z) is an electrical quantity that represents the capacity of a material to resist alternating current flow. When an electrical potential is applied to a media, the current flows through the intracellular and extracellular spaces at high frequencies (β-dispersion; 10 kHz–10 MHz) and extracellular spaces at low frequencies (α-dispersion; 10 Hz–10 kHz). On the other hand, the cell membrane acts as an insulator in low frequencies and acts as a conductor in high frequencies. Resistance (R) and reactance (X_C) are the components of Z, which is calculated in each frequency with $Z^2=X_C^2+R^2$. X_C is related to the capacitance that causes the phase shift that is defined by the phase angle (PA) and calculated by arctan (X_C/R). The current applied to the cell, flows through the extracellular fluid because cell membranes act as capacitors at low frequencies. Membrane capacitance increases with an increase in cell membrane area increases. At higher frequencies, this capacitive effect is lost, and then the current passes through the extracellular and intracellular fluid, and the resistance decreases (2).

Bioimpedance methods have also been used for tissue differentiation, identifying intraneural needle placement, and tumor detection (3-6). Studies have shown that bioimpedance can be used in the clinical setting for the differentiation of tissues. The small size required is challenging to produce bioimpedance sensing hypodermic needle; Kari et al. (7) presented a thin bioimpedance probe needle of standard 22 G size. In our study, we practiced the

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same technology; however, we used the 18 G 1.2×89 mm spinal needle, which is connected to the bioimpedance analyzer.

Bioimpedance was simultaneously measured with the probe that was tested to determine the bacteria types and colony numbers in CSF at 50 kHz PA and 5 kHz Z values. Our primary aim was to assess the performance of the examination in detecting the presence of bacteria in CSF *ex-vivo*. Our secondary objective was to examine the electrical differentiation with different colony numbers. We hypothesized that if the probe could detect the presence of bacteria in CSF accurately, it could be used as an early predictive diagnostic tool in laboratories.

METHODS

Study Design

The study was conducted at SANKO University School of Medicine, with the approval of the SANKO University Ethics Committee (2018/10-07). At the Medical Microbiology laboratory, a quantity of 200 µl sterile CSF was used as the standard and was inoculated with 1, 2, 5, and 10 colonies of Coagulase-negative staphylococci (CoNS), *Streptococcus pneumoniae (S. pneumoniae)* and *Acinetobacter baumannii (A. baumannii)*, separately. A minimum of five bioimpedance measurements (50 kHz PA and 5 kHz Z values) were taken from each specimen within 1–2 min and their mean values were examined.

Bioimpedance Measurements

A bioimpedance analyzer (Quadscan 4000, Bodystat Inc.) was connected to an 18 G probe, and the experimental setup is shown in Figure 1. Using the probe, current was sent to the CSF samples in multiple (5, 50, 100, and 200 kHz) frequencies for bioimpedance measurements. We used only 5 kHz Z values since this frequency is suitable for the cellular sizes and the current information from the outside of the cells was sufficient to differentiate the CSF samples. Moreover, R, X_C, and PA values were recorded at a 50 kHz frequency. Many bioimpedance systems use 50 kHz as a frequency where the capacitor's X_C becomes relatively small so that the current is defined mostly by the R. The frequency at 50 kHz is one of the most essential and optimal frequency, thus we obtained the PA in this frequency. Moreover, most published studies have been carried out using devices with a frequency of 50 kHz to differentiate structures. Due to the logic of this reasoning, we chose to illustrate our PA results only for 50 kHz. All bioimpedance signals were examined by IGOR program (Wavemetrics, Lake Oswego, OR, USA).

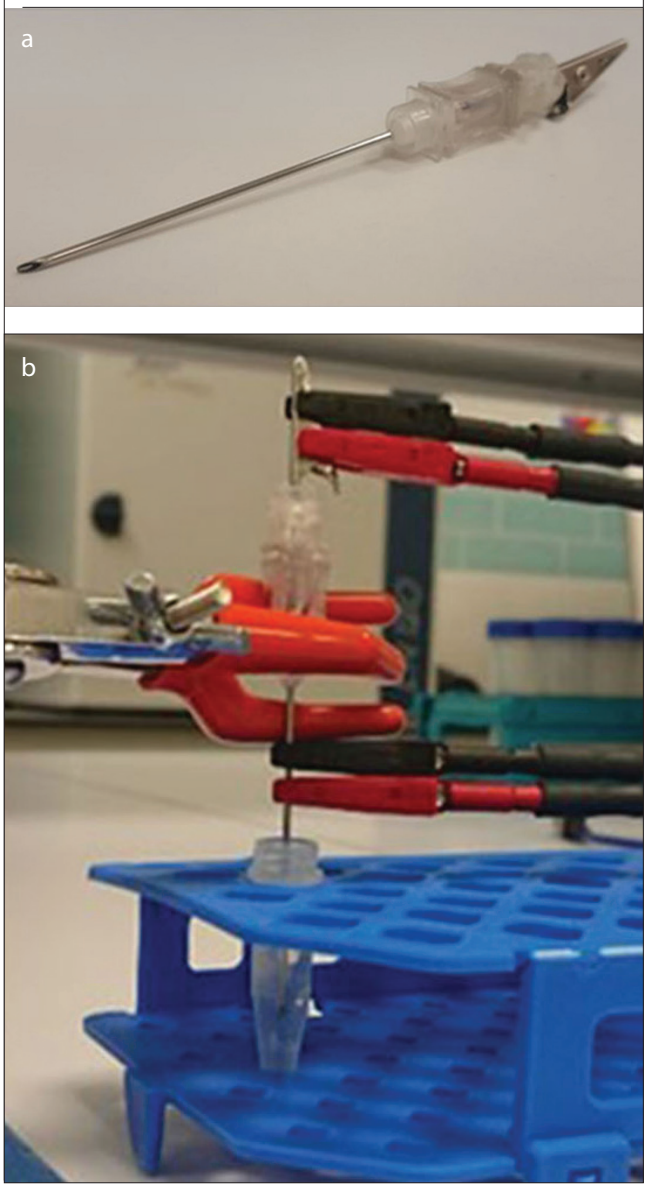
Suspension Preparation

Gram-negative *(A. baumannii)* and Gram-positive (CoNS and S. pneumoniae) bacterial strains which were isolated previously from various clinical samples were used. They were identified by the automated diagnostic system (Vitek2 Compact, Biomérieux, France) and were frozen at –80°C. CSF that was confirmed to be sterile after culture evaluation was stored at 2°C–8°C prior to use and was used as standard. Microorganisms were cultured onto 5% sheep blood agar (RTA Laboratories, Turkey) to prepare the suspension. The cultures were incubated at 37°C for 24 h in a CO_2 incubator, and then the microorganisms were transferred from plates to the sterile CSF. In our study, one colony contained 100 microorganisms (100 Colony-Forming Unit (CFU)/ml).

Main Points:

- Presence of bacteria and their colony numbers in CSF were investigated.
- Bioimpedance probe was time-saving and was able to detect the bacteria in CSF.
- 18 Gauge probe has the potential to be used during the real-time examination.

Figure 1. a, b. a) 18 G bioimpedance probe, b) Measurement set up



Statistical Analysis

IBM Statistical Package for the Social Sciences version 23.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analyses (8).

Figure 2. a-d. Impedance values of cerebrospinal fluid (CSF) samples at multiple frequencies with different colony numbers a) 1 colony, b) 2 colony, c) 5 colony and d) 10 colony (AB: *A. baumanni*; SP: *S. pneumoniae*)

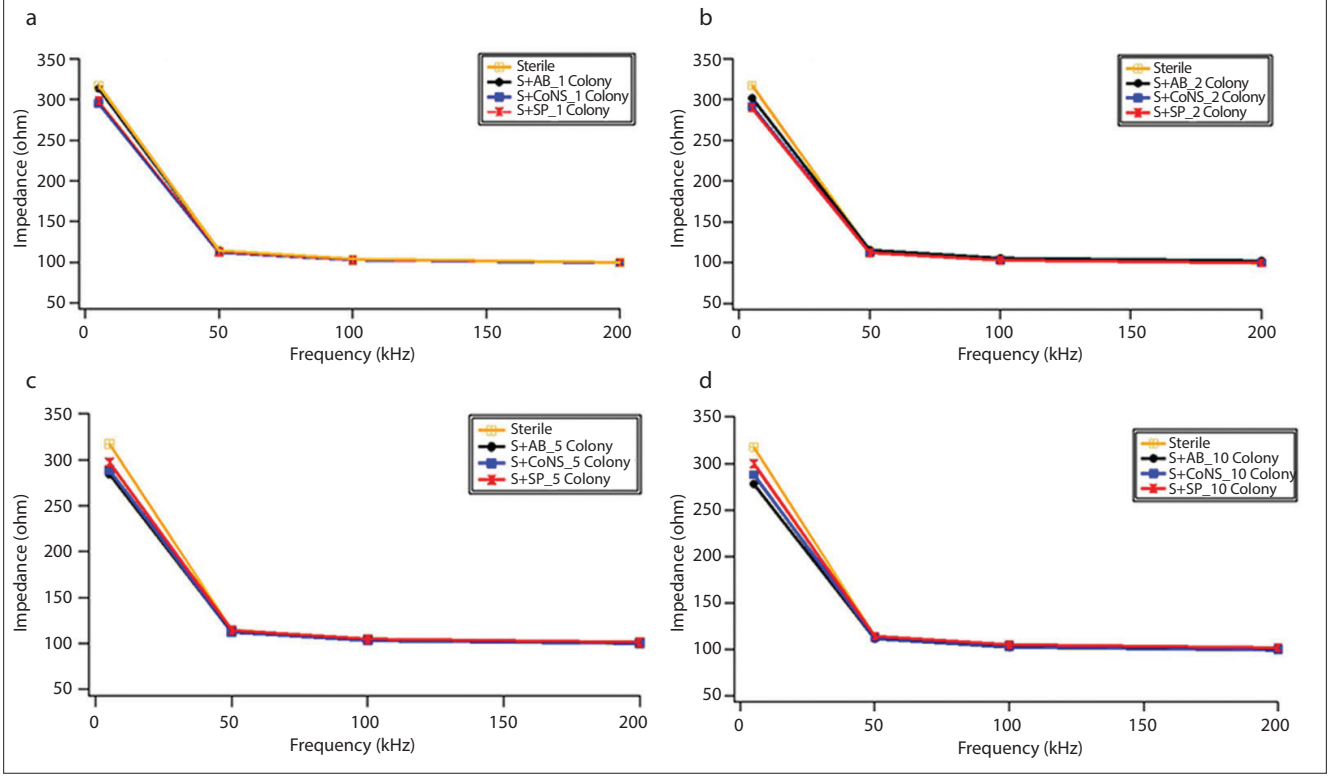


Table 1. Comparison of phase angle (PA) and impedance p–values for cerebrospinal fluid (CSF) samples at different colony numbers

p–values of PA and impedance at different colony numbers for *A. baumannii*, CoNS, S. pneumoniae and sterile CSF differentiation

Colony	PA	Impedance
1	0.001	0.001
2	0.001	0.0001
5	0.0001	0.0001
10	0.0001	0.0001

The Kruskal–Wallis test was used to compare the CSF samples according to PA at 50 kHz and impedance at 5 kHz. For pairwise comparisons, the Mann–Whitney U test was used with Bonferroni correction. A p- value <0.05 was considered to be statistically significant. The p-value was 0.013 for tests with Bonferroni correction.

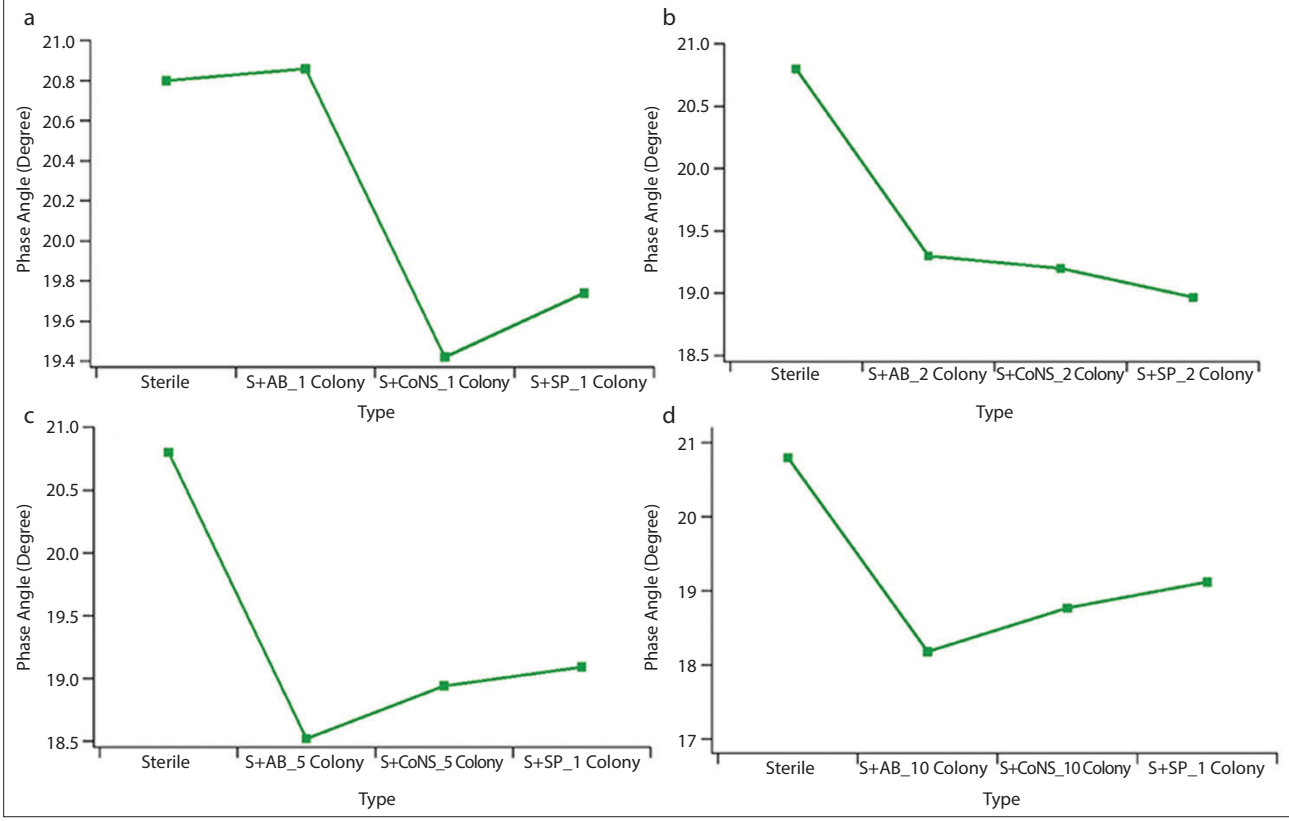
RESULTS

We found current at low frequency (5 kHz) exposed to a long resistive pathway around the one colony in CSF sample. However, the extracellular pathway decreased with the increase of colony number that caused a decrease in Z at low frequencies. In all the three cell types, Z was found high due to the strong dielectric characteristics of the cell membrane and the tissue interface acting as a capacitance in a low frequency (5 kHz). However, Z was low due to the loss of this capacitive effect of the membrane at high frequencies (50, 100, and 200 kHz) (Figure 2). The PA values of the sterile CSF were found higher than the non-sterile CSF with different colony numbers since the R values of non-sterile CSF samples were higher than sterile CSF (Figure 3). The values of PA at 50 kHz and the Z at 5 kHz obtained from different CSF samples were compared with each other (Table 1). The PA values of all CSF samples with different colony numbers were compared and it was observed that varying number of the colonies (1, 2, 5, 10) strains of *A. baumannii*, CoNS, and *S. pneumoniae* could be differentiated from sterile CSF by using their PA values. However, the sample with one colony of the *A. baumannii* strain could not be distinguished from sterile CSF by using PA value due to its thin cell wall composition (Table 2). Z values of all CSF samples were equally compared at different colony numbers. It was observed that varying numbers of the colonies (1, 2, 5, 10) strains of *A. baumannii*, CoNS, and S. pneumoniae could be differentiated from sterile CSF by using 5 kHz Z values (Table 2). However, the sample with one colony of the *A. baumannii* strain could not be distinguished from sterile CSF by using the 5 kHz Z value due to its thin cell wall composition.

DISCUSSION

According to the literature, the diameter of the *A. baumannii* cell was determined to be 0.9–1.6 µm (9), CoNS cell was defined

Figure 3. a-d. Phase angle (PA) values of different CSF samples at different colony numbers a) 1 colony, b) 2 colony, c) 5 colony and d) 10 colony (AB: *A. baumanni*; SP: *S. pneumoniae*)



as 0.5–1.5 µm in diameter (10), and *S. pneumoniae* cell wall was identified as 0.5–1.25 µm (11). Gram-positive (CoNS and *S. pneumoniae*) cell wall thickness is about 20–80 nm and more homogenous than that of the thin (2 nm) Gram-negative *(A. baumannii)* cell wall (12). The electrical characteristics of bacterial cells and their electrophysiology are fundamental for improving bioimpedance methods for the detection of bacterial cells. Like other biological cells, bacterial cells consist of structures that have various electrical features. The inner composition of a cell is sophisticated and contains vacuoles, mitochondria, nucleus, and many dissolved charged molecules. While the membrane of the cell is highly insulated, its interior is extremely conductive. The electrical conductivity of the cell membrane is about 10^{-7} S/m; however, the intracellular conductivity can be as high as 1 S/m. Bacterial suspension conductivity studies have investigated the electrical features of bacterial cell surface and related cell components (13).

Bioimpedance spectroscopy techniques have been used in some bacterial identification studies. In one study, Salmonella cell suspensions in deionized water solution were studied over a wide range of frequencies. It was reported that bacterial cell suspensions with different cell concentrations could result in various electrical Z spectral responses. It was stated that the Z of the cell suspension was related to the cell concentration which could present an alternative approach for quantifying bacterial cells in a label-free, cheap, and straightforward method (14). In another clinical study, it was demonstrated that the bioimpedance needle probe was a reliable tool for detecting spinal structures with a sensitivity of 100% and specificity 81% (15). In a certain study, 22 G and 24 G bioimpedance needle probes were employed for the detection of synovial fluid concentrations in the joints of 80 patients with active arthritis. It was found that the sensitivity and specificity of this probe for synovial fluid detection were 86% and 85% respectively (16). In another Z study, the authors demonstrated that the Z biosensor was capable of detecting Listeria as low as 1.6×10^2 CFU/mL in 1 h based on either the PA or the Z change analysis (17). In another study, *P. aeruginosa* was detected by electrical impedance spectroscopy (18). Among 262 CSF samples with positive cultures, the most frequently isolated agent was *S. pneumoniae* with 23%, which was followed by CoNS (21%), *A. baumannii* (10%), Neisseria meningitidies (9%), Enterobacteriaceae strains (9%), *Pseudomonas* spp. (7%), *Staphylococcus aureus* (5%), *Haemophilus influenzae* (3%), *Candida* spp. (2%), and *Enterococcus* spp. (2%) (19). Based on this study, we used the three most frequently isolated agents to compare with the sterile CSF in our research.

In studies that used PA to differentiate tissues, it was shown that a low PA was associated with tumor development, cell death or decreased cell integrity; however, high PA was associated with a healthy cell or cell membrane (20). Similar to these results, non-sterile CSF PA values were low and sterile PA values were high in our study.

Table 2. Pairwise comparison of phase angle (PA) and impedance values for cerebrospinal fluid (CSF) samples at different colony numbers

	p–values of PA/impedance at different colony numbers				
	A. baumannii	CoNS	*S. pneumoniae*	Sterile	Colony
A. baumannii	–				1
	–				2
	–				5
	–				10
CoNS	0.008/0.008	–			1
	1/0.008	–			2
	0.008/0.008	–			5
	0.003/0.003	–			10
S. pneumoniae	0.008/0.008	0.008/0.056	–		1
	0.052/0.004	0.004/0.537	–		2
	0.003/0.003	0.03/0.003	–		5
	0.001/0.001	0.0001/0.0001	–		10
Sterile	0.931/0.247	0.004/0.004	0.004/0.004	–	1
	0.004/0.004	0.004/0.004	0.002/0.002	–	2
	0.004/0.004	0.004/0.004	0.001/0.001	–	5
	0.004/0.004	0.001/0.001	0.0001/0.0001	–	10

We performed a rapid and straightforward bioimpedance method to differentiate sterile from non-sterile CSF with different colony numbers. In this study, we found that sterile and non-sterile CSF with different colony numbers resulted in various electrical Z spectral responses. However, the comparison of sterile CSF with CSF inoculated with *A. baumannii* did not yield any significant difference in Z spectra and PA in response to one colony due to its thin cell wall composition. The diameter cell membrane of *A. baumanni* is small due to its thin cell wall composition, and thus, the capacitive effect of its membrane is low. This situation may explain the fact that the cell membrane does not exhibit insulating properties and therefore has similar R results with the measurement from sterile CSF. Further, we observed that when colony numbers of *A. baumannii* were increased in CSF, the differentiation of sterile and non-sterile CSF became significant since the applied current interacted better with the inner structure of the *A. baumannii*. Similarly, its cell membrane did not exhibit insulating properties and therefore it had similar Z results with the measurement from sterile CSF. These results indicate that the bioimpedance probe differentiates the sterile from non-sterile CSF samples with different colony numbers.

CONCLUSION

Today, molecular tests have become prominent as rapid diagnostic methods in the diagnosis of meningitis. However, these tests are expensive and thus are not being used in routine practice. Bioimpedance spectroscopy technique can provide a predictive approach for quantifying bacterial cells in an inexpensive, simple, and time-saving way in CSF samples with different colony numbers. Besides, the probe has the potential to be used in the rapid detection of bacteria in CSF during real-time examinations.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of SANKO University (2018/10-07).

Informed Consent: Due to the design of the study, informed consent was not taken.

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