Since the emergence of microorganisms hinders the therapeutic application of antibiotics, investigation of microbial adaptation to antibiotics is an important biomedical problem. The effect of antibiotics may be due to a variety of factors, including the inhibition of cell wall synthesis, synthesis of proteins and/or RNA, and DNA replication, as well as impairment of the functioning of membranes [1]. In the presence of antibiotics, the morphology of microbial cells may be changed, their cellular membrane disrupted, and their cytoplasmic membrane modified; the biochemical processes occurring in these structures may therefore be impaired. This, in turn, may result in altered electrophysical (EP) characteristics of microbial cells and therefore in altered electrooptical (EO) characteristics of cell suspensions, which can be experimentally detected using the electrical orientation of the cells in an electric field. We have previously demonstrated the possibility of determining microbial resistance to ampicillin [2]. We believe that EO analysis of cell suspensions in the presence of antibiotics with an action mechanism different from that of ampicillin is a promising area of research. Inhibitors of protein synthesis, including aminoglycoside antibiotics, are an example. These antibiotics penetrate the outer membrane of gram-negative bacteria and displace magnesium ions from the external surface of the outer membrane; this process results in partial destruction of the membrane [3–4]. We expected that the membrane destruction caused by an antibiotic could change the EP characteristics of microbial cells; these, in turn, could be used as an indicator of microbial sensitivity to antibiotics under study.

The goal of the present work was to investigate the effect of kanamycin on the EO parameters of the cell suspensions of some *Escherichia coli* strains with different sensitivity to the antibiotic in question.

**MATERIALS AND METHODS**

**Microorganisms.** Strains *E. coli K-12* and *E. coli pMMB33* used in the present work were obtained from the strain collection of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (Saratov, Russia).

*E. coli K-12* and *E. coli pMMB33* were grown in a liquid medium containing the following (g/l): NaCl, 10; yeast extract, 5; and peptone, 5. The cultivation was carried out under aerobic conditions on a rotary shaker (160 rpm) at 30°C for 24 h. The cells were collected and used for EO studies.
Cell preparation for analysis. Prior to analysis, the cells were washed three times with 5-min centrifugation at 2800 g and resuspended in a small volume of distilled water (conductivity 1.8 µS/cm). To remove cell aggregates, the suspension was centrifuged for 1 min at 110 g; the supernatant was collected for the experiments. For every microbial strain, OD_{670} was adjusted to 0.4–0.42.

Orientation spectra of the cells were measured on an ELBIC electrooptical analyzer (State Scientific Center for Applied Microbiology, Obolensk, Moscow oblast) at 670 nm (relative to vacuum) according to [5]. The set of frequencies for the orienting electric field was as follows: 10, 52, 104, 502, 1000, 5020, and 10000 kHz. The orientation spectrum (OS) was represented as a frequency function of the difference between the OD values determined in nonpolarized light beams directed along and across the orienting field (δOD). This difference was normalized to the optical density obtained for chaotically oriented cells [6–8].

Microbial sensitivity to antibiotics was determined by the serial dilutions method [9]. The amount of an antibiotic in the test tube with visible growth inhibition was accepted as the minimal inhibiting concentration for the strain in question.

Antibiotic treatment. After the 24-h cultures of E. coli K-12 and E. coli pMMB33 were prepared for EO analysis, kanamycin (Sigma, United States) was introduced into the cell suspension (OD_{670} = 0.4–0.45). The suspension was incubated at 30°C for 5, 15, 30, 60, or 150 min. The cell suspension incubated for the same time at the same temperature without antibiotics was used as a control. After incubation, the cells were washed three times with distilled water (1.6–2.0 µS/cm) and used for EO measurements.

Colony count. In order to determine the number of colonies formed from individual viable cells, the standard plating method was used. The agarized medium contained the following (g/l): NaCl, 10; yeast extract (Fluka, Switzerland), 5; peptone (Fluka, Switzerland), 5. The cell suspension prepared for EO analysis was treated with kanamycin and incubated for 30 min at 37°C. The diluted suspension (0.1 ml) was applied to the surface of dried agar in petri dishes and spread with a glass spreading rod. After overnight incubation at 30°C, the number of colonies was determined under adequate illumination. The colony counts obtained without antibiotic treatment were used as the control [10].

RESULTS AND DISCUSSION

Kanamycin is an aminoglycoside antibiotic of the oligosaccharide group. It operates mainly by disrupting protein synthesis at the stage of amino acid transfer from aminoacyl-tRNA to the ribosome. Kanamycin promotes binding with the ribosomes of those aminoacyl-tRNAs that do not correspond to the codon of the ribosomal A site. Due to such faulty encoding, polypeptides with numerous errors are synthesized; a cytotoxic (bactericidal) effect results [11]. Since this antibiotic is active against various gram-negative rods, E. coli was chosen as an object of our study. The experiment was aimed at comparative study of electrooptical characteristics of microbial cells with different sensitivity to an antibiotic.

At the first stage, the electrooptical characteristics of the cell suspension of the kanamycin-sensitive strain E. coli K-12 was studied under different kanamycin concentrations (0.5, 1.0, 2.0, 5.0, 7.0, 10, 15, and 20 µg/ml). The changes in OS of the K-12 cell suspensions were revealed at frequencies of the orienting electric field within the range of 10–1000 kHz. No considerable changes were detected at higher frequencies. For more convenient representation of the experimental data, we present the EO signal obtained at an orienting field of 52 kHz. The data presented in Fig. 1 (curve 1) demonstrate that addition of the above antibiotic concentrations resulted in a gradual decrease in the EO signal, with the minimal value at 10 µg/ml kanamycin. The mechanism of antimicrobial action of kanamycin is related to suppressed protein synthesis with the subsequent inhibition of nucleic acid synthesis and disrupted cell wall formation. Aminoglycosides penetrate the outer membrane of gram-negative bacteria via porin channels; the membrane is partially disrupted, and ami-