

Rapid Antimicrobial Susceptibility Testing by Sensitive Detection of Precursor rRNA Using a Novel Electrochemical Biosensing Platform

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Precursor rRNA (pre-rRNA) is an intermediate stage in the formation of mature rRNA and is a useful marker for cellular metabolism and growth rate. We developed an electrochemical sensor assay for *Escherichia coli* pre-rRNA involving hybridization of capture and detector probes with tail sections that are spliced away during rRNA maturation. A ternary self-assembled monolayer (SAM) prepared on gold electrode surfaces by coassembly of thiolated capture probes with hexanedithiol and posttreatment with 6-mercaptop-1-hexanol minimized the background signal and maximized the signal-to-noise ratio. Inclusion of internal calibration controls allowed accurate estimation of the pre-rRNA copy number per cell. As expected, the ratio of pre-rRNA to mature rRNA was low during stationary phase and high during log phase. Pre-rRNA levels were highly dynamic, ranging from 2 copies per cell during stationary phase to ~1,200 copies per cell within 60 min of inoculation into fresh growth medium. Specificity of the assay for pre-rRNA was validated using rifampin and chloramphenicol, which are known inhibitors of pre-rRNA synthesis and processing, respectively. The DNA gyrase inhibitor, ciprofloxacin, was found to act similarly to rifampin; a decline in pre-rRNA was detectable within 15 min in ciprofloxacin-susceptible bacteria. Assays for pre-rRNA provide insight into cellular metabolism and are promising predictors of antibiotic susceptibility.

There is an urgent need for the development of rapid and convenient methods for detection and identification of bacterial pathogens in clinical specimens to guide the diagnosis and treatment of infectious diseases. Electrochemical biosensors are molecular-sensing devices that intimately couple a biological recognition element to an electrode transducer. For example, the widely used handheld glucose sensors link substrate-dependent activity of glucose oxidase enzymes to an amperometric current readout (1). Similarly, electrochemical sensor assays are able to measure the number of DNA capture probe-target hybridization events on the sensor surface through the catalytic rate of redox enzymes linked to detector probes (2). This capture probe-target-detector probe sandwich assay approach has been adapted to species-specific identification of a broad array of bacterial pathogens using capture and detector probe combinations targeting rRNA (3–8), an approach which has been validated on human clinical urine specimens from patients with urinary tract infection (3, 5).

rRNA is an excellent target molecule for pathogen detection systems because of its abundance in the bacterial cell and because of the accessibility of species-specific signature sequences to probe hybridization (9). When combined with sensitive surface chemistry methods to minimize nonspecific background signals, such rRNA probe hybridization sensors are able to detect as few as 100 bacteria per milliliter (10–12). Estimations of bacterial density are possible because, within the dynamic range of the assay, there is a log-log correlation between the concentration of target rRNA molecules in the bacterial lysate and the amperometric current amplitude generated by the electrochemical sensor assay (4, 6). The accuracy of bacterial quantitation methods based on rRNA detection is mitigated by variations in the number of rRNA molecules per cell depending on the cell type and bacterial growth phase. In *Escherichia coli*, the rRNA copy number per cell has been

estimated to vary from as high as 72,000 during log phase to less than 6,800 during stationary phase (13).

Electrochemical sensors have the potential to rapidly determine antibiotic susceptibility by monitoring the phenotypic response of bacteria to antibiotics. Cellular precursor rRNA (pre-rRNA) levels would be expected to fall as antibiotics shift the cellular metabolism of antibiotic-susceptible bacteria from log phase to stationary phase. The size of the pre-rRNA pool in the cell is determined by the synthesis and degradation rates, which are directly or indirectly affected by antibiotics (14). For this reason, we developed and validated an electrochemical assay for pre-rRNA determination. By calibrating sensor signal intensities with an internal standard, and correlating these signals with bacterial density, we were able to estimate the number of rRNA and pre-rRNA copies per cell. Our studies provide new insight into the kinetics of rRNA and pre-rRNA levels during bacterial growth phases, and in response to certain antibiotics. Of interest, we determined that pre-rRNA and/or rRNA levels rapidly respond to the quinolone antibiotic, ciprofloxacin, and the aminoglycoside antibiotic, gentamicin, in susceptible *E. coli*.

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MATERIALS AND METHODS

Bacterial strains and media. *E. coli* clinical urine isolate EC103 (Amp^r) was obtained from the University of California—Los Angeles (UCLA) Clinical Microbiology Laboratory with approval from the UCLA and Veterans Affairs Institutional Review Boards and appropriate Health Insurance Portability and Accountability Act exemptions. EC103 was inoculated into Mueller-Hinton (MH) broth with 12% glycerol (Becton, Dickinson, Sparks, MD) and stored at -80°C . EC103 was cultured overnight in MH broth with 64 $\mu\text{g}/\text{ml}$ ampicillin (Sigma, St. Louis, MO). EC103 was plated on Luria broth (LB) agar (MOBIO Laboratories Inc., Carlsbad, CA) for counting CFU.

EC103 growth and target copy number experiments. Overnight cultures of EC103 were prepared by adding 5 μl of EC103 glycerol stock to 5 ml of MH broth with ampicillin and incubated at 37°C overnight, with shaking. The following day, the EC103 culture was diluted by adding 10 μl of the overnight culture to 100 ml of prewarmed and preshaken MH broth in a 500-ml flask, followed by incubation at 37°C , with shaking at 250 rpm. Every 30 min, including at 0 min and during the overnight culture itself, a 1-ml sample was taken for a measurement of an optical density at 600 nm (OD_{600}) and 10-fold serial dilutions (100 μl into 900 μl) were performed in room temperature MH broth. Cell density was determined by plating serial dilutions in triplicate. At each time point, culture samples were transferred to an ice-water bath or centrifuged immediately at 4°C for 3 min at 14,000 rpm. The supernatants were then removed by aspiration, flash frozen in a dry ice-ethanol bath and stored at -80°C .

In certain growth experiments, one culture was spiked with one of the following antibiotics at either 150 or 210 min: 25 $\mu\text{g}/\text{ml}$ rifampin (Sigma, St. Louis, MO), 25 $\mu\text{g}/\text{ml}$ chloramphenicol (Sigma, St. Louis, MO), 4 $\mu\text{g}/\text{ml}$ ciprofloxacin (Sigma, St. Louis, MO), or 16 $\mu\text{g}/\text{ml}$ gentamicin (Sigma, St. Louis, MO). After the addition of antibiotics at 150 min, samples were collected every 15 min instead of every 30 min.

For experiments comparing preribosomal probe sensitivity and specificity, culture samples were taken from the overnight culture and the EC103 culture at log phase ($\text{OD}_{600} = 0.1$) and centrifuged immediately at 4°C for 5 min at 14,000 rpm. Supernatants were removed by aspiration. The pellets were flash frozen in a dry ice-ethanol bath and stored at -80°C .

Electrochemical detection. Electrochemical detection of bacterial rRNA and pre-rRNA was performed as described previously for biotinylated (6) and thiolated capture probes (10, 11) immobilized on photolithographically prepared Au electrode arrays, with modifications.

The sensor response was evaluated with a sandwich-type hybridization assay, using fluorescein (FITC) as a tracer in the detector probe and anti-FITC-horseradish peroxidase (anti-FITC-HRP) as the reporter molecule. 3,3',5,5'-tetramethylbenzidine (TMB)- H_2O_2 was the selected substrate for the electrochemical measurement of the activity of the captured HRP reporter. All synthetic oligonucleotides used were purchased from Eurofins MWG Operon and are listed in Table 1. For thiolated capture probes, disposable 16-sensor bare Au electrode arrays were obtained from GeneFluidics (Irwindale, CA). Each sensor of the array consisted of a 2.5-mm diameter central working electrode, surrounded by an Au counter electrode and an Au pseudo-reference electrode. The sensor chip was driven by a computer-controlled Helios multichannel electrochemical workstation (GeneFluidics, Irwindale, CA). Washing steps were carried out after each application of reagents by applying a stream of deionized H_2O to the sensor surface for approximately 2 to 3 s, followed by 5 s of drying under a stream of nitrogen. Prior to the addition of the first reagent, the bare gold chips were dried as described above. To functionalize the working sensor surface, a fresh mixture of 0.05 μM thiolated capture probe and 300 μM 1,6-hexanedithiol (96%; Sigma, St. Louis, MO) was prepared in 10 mM Tris-HCl, 1 mM EDTA, and 0.3 M NaCl (pH 8.0) and allowed to stand at room temperature for 10 min. Aliquots of 6 μl of this mixture were cast over each Au working electrode in the 16-sensor array and incubated overnight at 4°C in a humidified chamber. Unless otherwise stated, all subsequent steps were performed at room temperature.

TABLE 1 DNA oligonucleotides used in this study

Probe name ^a	Sequence ^b
Pre16S 15 m 48D	5'-TTTTTCGTCTTGCGA-F
Pre16S 15 m 63C	5'-B-GAGACTTGGTATTCA
Pre16S 15 m R63D	5'-F-GAGACTTGGTATTCA
Pre16S 15 m R48C	5'-TTTTTCGTCTTGCGA-B
Pre16S 17 m R63D	5'-F-TTGAGACTTGGTATTCA
Pre16S 17 m R46C	5'-TTTTTCGTCTTGCGACG-B
Pre16S 19 m R63D	5'-F-TCTTGAGACTTGGTATTCA
Pre16S 19 m R44C	5'-TTTTTCGTCTTGCGACGTT-B
Pre16S 21 m R63D	5'-F-ACTCTTGAGACTTGGTATTCA
Pre16S 21 m R42C	5'-TTTTTCGTCTTGCGACGTTAA-B
Pre16S 17 m R60D	5'-F-AGACTTGGTATTTCATTT
Pre16S 17 m R43C	5'-TTCGTCTTGCGACGTTA-B
Pre16S 19 m R60D	5'-F-TGAGACTTGGTATTTCATTT
Pre16S 19 m R41C	5'-TTCGTCTTGCGACGTTAAG-B
Pre16S 21 m R60D	5'-F-CTTGAGACTTGGTATTTCATTT
Pre16S 21 m R39C	5'-TTCGTCTTGCGACGTTAAGAA-B
Pre16S 17 m R66D	5'-F-CTCTTGAGACTTGGTAT
Pre16S 17 m R49C	5'-TCATTTTTTCGTCTTGCG-B
Pre16S 19 m R66D	5'-F-CACTCTTGAGACTTGGTAT
Pre16S 19 m R47C	5'-TCATTTTTTCGTCTTGCGAC-B
Pre16S 21 m R66D	5'-F-TTCACCTCTTGAGACTTGGTAT
Pre16S 21 m R45C	5'-TCATTTTTTCGTCTTGCGACGT-B
Pre16S 19 m 5'JxnD	5'-TTTGATGCTCAAAGAATTA-F
Pre16S 21 m 5'JxnC	5-S-TCAAACCTCTTCAATTTAAAAG
Pre16S 21 m R5'JxnD	5-F-TCAAACCTCTTCAATTTAAAAG
Pre16S 19 m R5'JxnC	5'-TTTGATGCTCAAAGAATTA-S
Pre16S 17 m 3'JxnD	5'-GAGGTGATCCAAACCGCA-F
Pre16S 20 m 3'JxnC	5-S-GAACGCTTCTTTAAGGTAAG
Pre16S 20 m R3'JxnD	5-F-GAACGCTTCTTTAAGGTAAG
Pre16S 17 m R3'JxnC	5'-GAGGTGATCCAAACCGCA-S
Pre23S 17 m 3'JxnD ^c	5'-AAGCCTCACGGTTCATT-F
Pre23S 14 m 3'JxnC ^c	5'-S-GGCGTTGTAAGGTT
Pre23S 14 m R3'JxnD	5'-F-GGCGTTGTAAGGTT
Pre23S 17 m R3'JxnC	5'-AAGCCTCACGGTTCATT-S
Mature rRNA 18 m 1484D	5'-GTTACGACTTCACCCAG-F
Mature rRNA 19 m 1502C	5'-S-GTCCCTACGGTTACCTT
Synthetic target oligonucleotides	
Pre-rRNA 31 m	5'-AATGAACCGTGAGGCTTAAC CTTACAACGCC
Mature rRNA 37 m	5'-CTGGGGTGAAGTCGTAACA AGGTAACCGTAGGGGAAC

^a m, number of nucleotides; C, capture probe; D, detector probe; Jxn, splice site; R, reverse orientation.

^b F, FITC; B, biotin; S, thiol.

^c Capture and detector probe pair selected based on its high signal-to-noise ratio.

The following day, the mixed monolayer-modified Au sensors were subsequently treated with 6 μl of 1 mM 6-mercapto-1-hexanol (97%; Sigma, St. Louis, MO) in 10 mM Tris-HCl, 1 mM EDTA, and 0.3 M NaCl (pH 8.0) for 50 min to obtain the ternary monolayer interface.

For biotinylated capture probes, 16-sensor Au electrode arrays pre-capped with a binary self-assembled monolayer (SAM) consisting of mercaptohexanol and mercaptoundecanoic acid in a 5:1 ratio were obtained from GeneFluidics (Irwindale, CA). Washing steps were carried out after each application of reagents by applying a stream of deionized H_2O to the sensor surface for approximately 2 to 3 s, followed by 5 s of drying under a stream of nitrogen. To functionalize the sensor surface, the carboxylic terminal groups of the binary SAM were converted to amine-reactive esters by applying 4 μl of a NHS/EDC (50 mM *N*-hydroxysuccinimide, 200 mM *N*-3-dimethylaminopropyl-*N*-ethylcarbodiimide; Sigma, St. Louis, MO) solution in deionized H_2O to the working electrode for 10

TABLE 2 Pre-rRNA and mature rRNA quantitation during *E. coli* growth phases

Time point (min)	OD ₆₀₀	CFU/ml	Rate ^a	Doubling ^b	Generation time (min)	rRNA ^c	Pre-rRNA ^c	Ratio	Growth phase
Overnight culture	2.307	6.67E + 09				6,009	2	2,720	Stationary phase
0	0	5.90E + 05				5,942	110	54	Lag phase
30	0	5.70E + 05				28,427	457	62	
60	0.003	8.63E + 05	0.18	1.20	50.08	55,278	696	79	Log phase
90	0.008	2.30E + 06	0.43	2.83	21.19	78,741	970	81	
120	0.024	6.20E + 06	0.43	2.86	21.00	91,049	1200	76	
150	0.068	1.91E + 07	0.49	3.24	18.51	98,782	523	189	
180	0.175	6.53E + 07	0.53	3.55	16.88	61,571	235	262	
210	0.409	2.30E + 08	0.55	3.64	16.50	38,033	208	183	
240	0.707	5.27E + 08	0.36	2.39	25.14	29,801	114	260	Early stationary phase
270	1.095	1.17E + 09	0.35	2.30	26.14	19,610	42	466	
300	1.466	2.33E + 09	0.30	2.00	30.00	13,162	25	536	
330	1.686	3.23E + 09	0.14	0.94	63.74	7,608	11	665	Stationary phase
360	1.834	4.43E + 09	0.14	0.91	65.87	5,734	8	723	
390	1.957	5.03E + 09	0.06	0.37	163.81	4,899	7	706	
420	2.051	5.73E + 09	0.06	0.38	159.68	6,230	6	1,049	

^a Growth rate in log units per 30 min.

^b Doublings per hour.

^c Copies per cell.

(Fig. 1). These target sequences are digested into two pieces during processing of pre-rRNA into mature rRNA such that after digestion, neither piece of the target sequence would bind the probe sufficiently well to generate a signal. Probe pairs were tested for binding to the 5' and 3' splice sites of 16S rRNA and the 3' splice site of 23S rRNA. Probe pairs in both the capture-detector and detector-capture orientations were tested. As shown in Fig. 2, pre-rRNA probe pairs targeting the splice sites resulted in higher ratios of log- to stationary-phase signals. These results are consistent with those of Cangelosi et al. (14), who used a pre-rRNA sandwich hybridization assay in which their capture probe bound to the pre-rRNA tail and their detector probe bound to the mature rRNA region, providing specificity for intact pre-rRNA. One of the two probe pairs for the 3' splice site of 23S rRNA produced a high signal with a relatively high signal ratio for log-phase cells compared to stationary-phase cells. This capture (Pre23S 14m 3'JxnC) and detector (Pre23S 17m 3'JxnD) probe pair was selected for subsequent measurements of pre-rRNA.

For mature rRNA determination, capture and FITC detector probes specified in Table 1 were used.

Growth phase comparison of mature rRNA versus pre-rRNA. We compared signals for mature rRNA versus pre-rRNA for an overnight culture of *E. coli* before and after inoculation into fresh MH medium. Target rRNA and pre-rRNA concentrations were estimated by including known concentrations of synthetic artificial target oligonucleotides as internal calibration controls on each electrochemical sensor chip. These synthetic target oligonucleotides functioned by hybridizing with both the capture and detector probes. Copies per cell were calculated from the concentrations of the rRNA target number and the number of cells in the bacterial lysate. We found that variability in pre-rRNA and rRNA measurements could be reduced by chilling samples in an ice bath and centrifugation in a centrifuge refrigerated at 4°C. On the other hand, the cells were sensitive to cold shock, particularly during the lag and early log phases of growth. For this reason, accurate plate counts were obtained by dilution of the culture in room temperature medium rather than cold medium.

Immediately after inoculation of overnight culture into fresh growth medium (Table 2, time zero), there was a 55-fold increase in pre-rRNA from 2 copies per cell to 110 copies per cell, indicating a dramatic induction of rRNA synthesis. At that point, the ratio of mature rRNA to pre-rRNA reached a nadir of 54:1. As shown in Fig. 3, pre-rRNA levels continued to increase during the first 2 h of incubation, peaking at 120 min after incubation at 1,200 copies per cell. As pre-rRNA was converted to mature rRNA, copies of mature rRNA peaked at >98,000 copies per cell at 150 min after inoculation. Despite a gradual drop in both mature rRNA and pre-rRNA thereafter, the growth rate peaked at 210 min at 1.1-log-unit increases in cellular concentration per hour, which equals a doubling time of 16.5 min. During the later phases of growth, pre-rRNA copy numbers dropped more quickly than mature rRNA copy numbers, eventually leading to an increase in the ratio of mature rRNA to pre-rRNA of >1,000:1.

As shown in Fig. 4A, there was a good correlation between cell volume and rRNA copies per cell during log and late log phases of growth, indicating a relatively constant rRNA density in the cytoplasm. This correlation was lost at cell densities above an OD₆₀₀ of 1.0, at which point the cell volume stabilized while the rRNA copy number continued to fall. Cryo-electron microscopy was performed to measure *E. coli* cell volumes at different growth phases. As shown in Fig. 4B, *E. coli* cells became progressively shorter and thinner as cells went from log phase to stationary phase. The peak average cell size was 2.8 μm³ (4.87 μm long by 0.85 μm wide), and the smallest average cell size was 0.45 μm³ (1.35 μm long by 0.65 μm wide).

Effects of antibiotics on mature rRNA and pre-rRNA levels. To confirm that the pre-rRNA capture and detector probes were selective for the desired target, we examined the effects of rifampin and chloramphenicol on pre-rRNA levels relative to mature rRNA. Consistent with a previous report (14), addition of rifampin caused a selective drop in pre-rRNA, while chloramphenicol caused a selective increase in pre-rRNA (Fig. 5A and B). The effects of ciprofloxacin and gentamicin on pre-rRNA and mature rRNA levels were also examined. As shown in Fig. 5C, ciprofloxacin

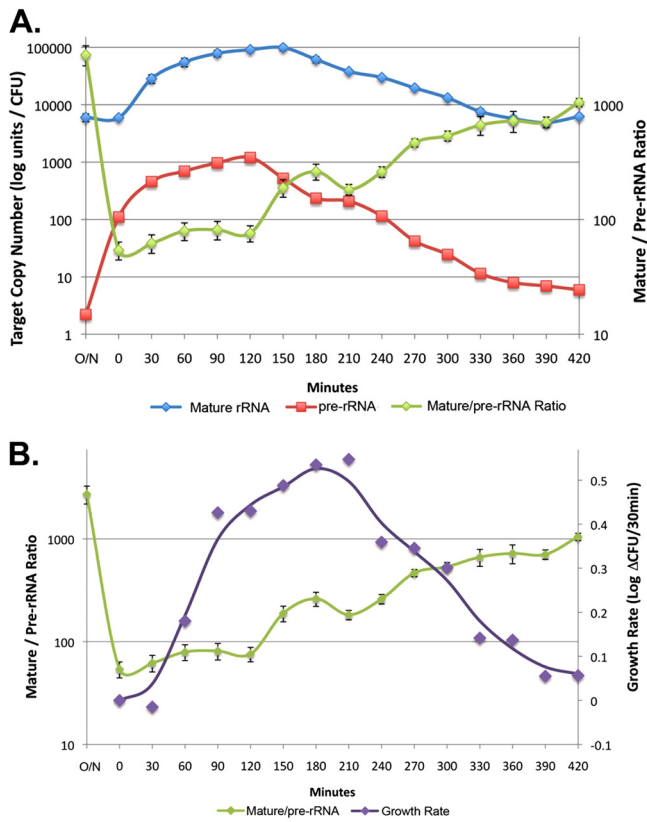


FIG 3 Variations in pre-rRNA and rRNA levels during *E. coli* growth. (A) Changes in mature rRNA, pre-rRNA, and their ratio were measured in overnight cultures that were subsequently inoculated into fresh MH growth medium and incubated for 7 h at 37°C. (B) Comparison of the mature/pre-rRNA ratio and growth rate during different phases of growth. The growth rate curve is a weighted average determined from the change in CFU during each 30-min time period. Error bars estimated the standard deviations.

cin had an effect similar to that of rifampin; pre-rRNA levels dropped significantly within 15 min while mature rRNA remained at control levels until 45 min after addition of the antibiotic. In contrast, there was no effect of this antibiotic on the pre-rRNA levels of ciprofloxacin-resistant organisms (Fig. 6). Addition of gentamicin resulted in a decrease in mature rRNA without affecting the level of pre-rRNA (Fig. 5D).

DISCUSSION

In this report, we describe an electrochemical sensor assay for detection and quantitation of pre-rRNA. Pre-rRNA represents a labile pool of rRNA precursor molecules produced during rRNA transcription. Pre-rRNA differs from mature rRNA by the presence of 5' and 3' tails that are removed during the maturation process. Because pre-rRNA represents a relatively small fraction (0.1% to 10%) of total rRNA, a sensitive assay is required for its detection. To achieve the needed sensitivity, our electrochemical Au sensor assay relies on the use of a ternary interface involving hexanedithiol coimmobilized with a thiolated capture probe, followed by the incorporation of 6-mercapto-1-hexanol as diluent. This new interface has been shown to offer a greatly improved surface blocking and maximal hybridization efficiency allowing ultrasensitive electrochemical detection of target nucleic acids (10–12). Direct nucleic acid detection methods, such as our electrochemical sandwich hybridization assay, have inherent advantages over methods that require target amplification, such as quantitative reverse transcription-PCR (qRT-PCR). We were able to quantitate pre-rRNA during different *E. coli* growth phases and documented dramatic shifts in copy number, from 2 to 1,200 copies per cell in the stationary and log phases of growth, respectively. To our knowledge, this is the first time that pre-rRNA copy numbers per cell have been quantitated electrochemically. The 600-fold increase in pre-rRNA copy number that we observed is considerably larger than the 50-fold increase reported by Cangelosi et al. (14) using luminescence detection. Possible reasons

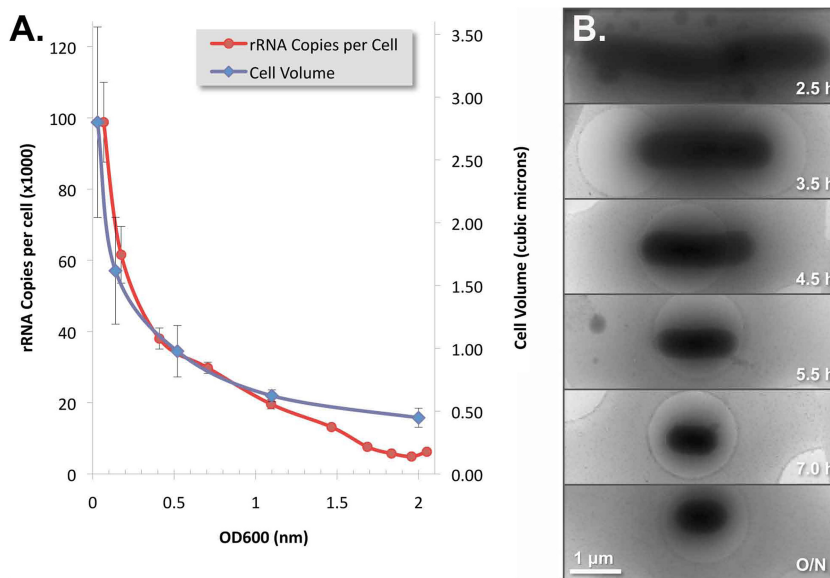


FIG 4 *E. coli* cell volume versus rRNA copy number during different growth phases. (A) Correlation between the cell volume and rRNA copy number per cell at densities below an OD₆₀₀ of ≤1.0. (B) Electron micrographs demonstrating progressively smaller cells over incubation time from log phase (2.5 h) to stationary phase (7 h). Error bars estimated the standard deviations.

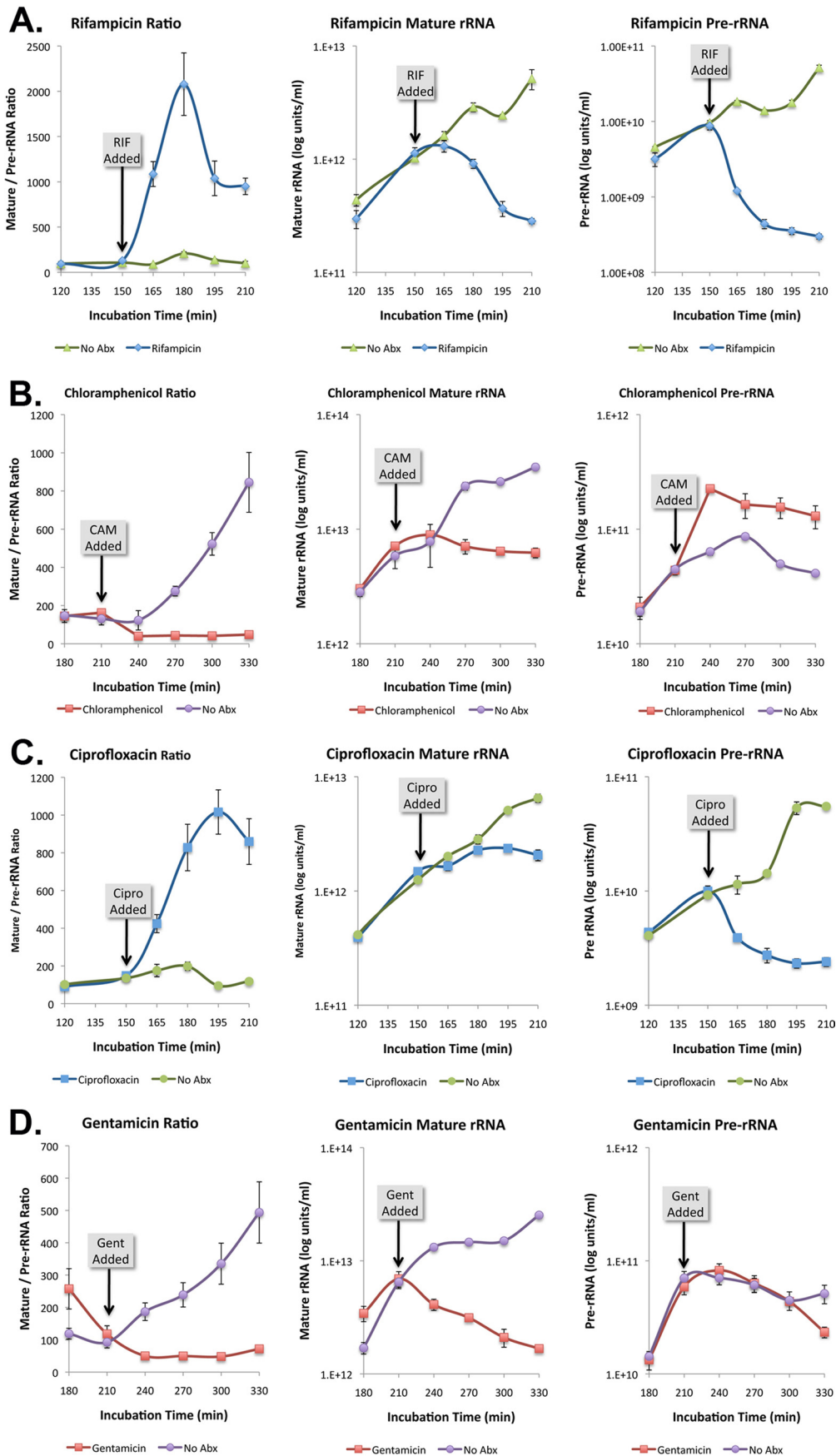


FIG 5 (A to D) Response of mature rRNA and pre-rRNA to antibiotics. Antibiotics have differential effects on rRNA and pre-rRNA. Rifampin and ciprofloxacin selectively inhibited transcription of new pre-rRNA, while the addition of chloramphenicol and gentamicin resulted in a selective decrease in mature rRNA. Error bars estimated the standard deviations.

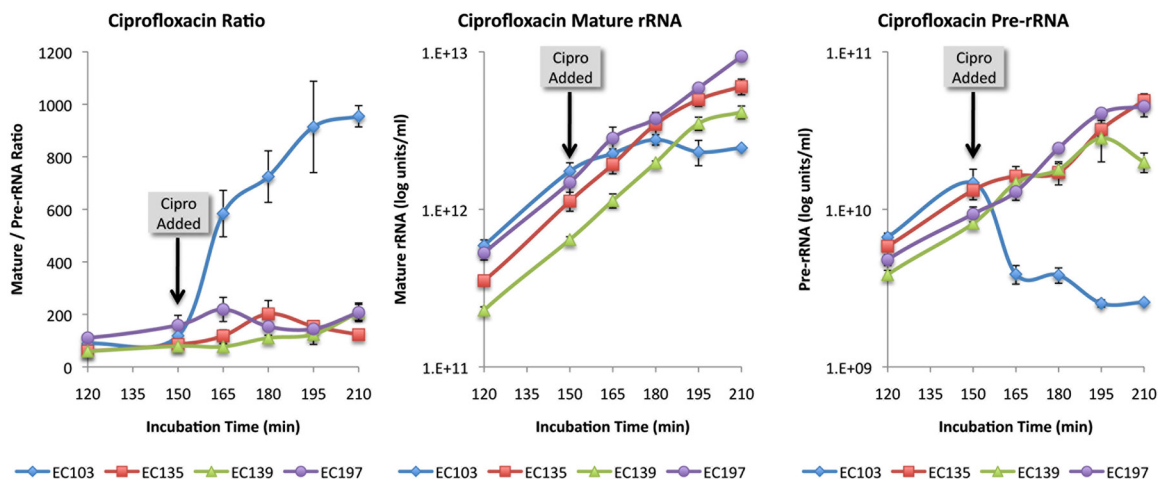


FIG 6 Comparison of ciprofloxacin-susceptible and -resistant *E. coli* strains. rRNA and pre-rRNA were measured in cultures of an *E. coli* clinical isolate susceptible to ciprofloxacin (EC103) and three ciprofloxacin-resistant isolates (EC135, EC139, and EC197). The amount of pre-rRNA in strain EC103 was significantly lower than that of the ciprofloxacin-resistant isolates within 15 min after addition of the antibiotic. Error bars estimated the standard deviations.

for this difference include a low limit of detection and the *E. coli* strain type. Cangelosi et al. examined the *E. coli* type strain ATCC 11775, which was isolated in 1895 by Migula and may have undergone metabolic changes during passage. In contrast, our studies were performed on a recently isolated wild-type uropathogenic *E. coli* strain with a relatively fast peak doubling time of 16.5 min.

Antibiotics differ in their effects on pre-rRNA and mature rRNA. Rifampin is an inhibitor of prokaryotic DNA-dependent RNA polymerase. Because pre-rRNA is rapidly processed to mature rRNA, inhibiting transcription quickly reduces the pool of pre-rRNA, especially during the log phase of growth. In contrast, chloramphenicol and gentamicin are protein synthesis inhibitors. Chloramphenicol acts by binding to the 23S subunit of bacterial ribosomes to inhibit protein synthesis, whereas gentamicin acts by inhibiting the proofreading function of ribosomes, thereby introducing translation errors and premature peptide chain termination events. In either case, these protein synthesis inhibitors should not directly interfere with pre-rRNA synthesis. Accordingly, we observed a decrease in the pool of mature rRNA, presumably because of the loss of proteins required for ribosome formation and stability. In the case of chloramphenicol, inhibition of pre-rRNA processing resulted in not only a decrease of mature rRNA but an increase in pre-rRNA (Fig. 5B).

Ciprofloxacin is a quinolone antibiotic that inhibits the activity of DNA gyrase, the bacterial topoisomerase that introduces and relaxes DNA supercoils. Relaxing of supercoils is required for unpackaging of DNA prior to not only DNA replication but also RNA transcription (15). As in the case of rifampin, inhibition of RNA transcription by ciprofloxacin resulted in a rapid decrease in pre-rRNA, detectable within 15 min after addition of the antibiotic. Quinolone resistance typically results from gyrase mutations that prevent binding of the quinolone to the gyrase. As expected, addition of ciprofloxacin had no discernible effect of pre-rRNA levels in ciprofloxacin-resistant organisms (Fig. 6).

There is a considerable interest in methods for determining the susceptibility of bacteria in clinical specimens in a time frame sufficient to impact clinical decision making. A major drawback of current clinical bacteriology methods is the need to isolate bacteria on solid agar media when processing a clinical specimen. In the

absence of expeditious antibiotic susceptibility testing, clinicians typically initiate “empirical” antibiotic treatment, meaning that antibiotics are chosen based on prior knowledge of potential organisms and their antibiotic resistance patterns. Empirical antibiotics for bacteremia are typically broad-spectrum to treat a wide variety of possible bacterial pathogens. This approach is especially problematic in the management of complex urinary tract infections where quinolone resistance rates are typically 20 to 30% (16). In addition, overuse of broad-spectrum antibiotics contributes to the emergence of antibiotic resistance by applying selective pressure to the patient’s flora and favoring colonization by resistant organisms.

To address the need for antibiotic resistance data at the time of initial antibiotic selection, methods are needed to analyze the antibiotic susceptibility of organisms in clinical specimens. The electrochemical sensor assay has been validated on human clinical urine specimens from patients with urinary tract infection (3, 5). Electrochemical sensor assays for pre-rRNA would be expected to be useful for identifying bacteria that are susceptible to antibiotics such as rifampin and ciprofloxacin that directly or indirectly inhibit RNA transcription. It may be possible to extend this approach for antibiotic susceptibility testing to other drugs by first depleting pre-rRNA levels and then measuring the ability of the antibiotic to inhibit pre-rRNA replenishment (17). However, because antibiotics act by widely divergent mechanisms, various approaches may be necessary to achieve comprehensive antibiotic susceptibility testing. For example, we have successfully applied ATP bioluminescence to determine antimicrobial susceptibility of uropathogens within 120 min after inoculation of clinical urine specimens into growth medium with and without antibiotics (18). Application of such assays to bacteria in clinical specimens at the point of care would enable patient-specific antibiotic therapy.

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