Biosensors and Bioelectronics 135 (2019) 200–207

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Direct antimicrobial susceptibility testing of bloodstream infection on SlipChip

Qiaolian Yi[a,d,1], Dongyang Cai[a,c,1], Meng Xiao[b], Mengyue Nie[a,d], Qinna Cui[a,d], Jingwei Cheng[b], Caiming Li[a,d], Jie Feng[a,d], Gerald Urban[c], Ying-Chun Xu[b,***], Ying Lan[a,**], Wenbin Du[a,d,e,*]

[a] State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China
[b] Department of Clinical Laboratory, Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100101, China
[c] Department of Microsystems Engineering (IMTEK), University of Freiburg, Freiburg, 79110, Germany
[d] College of Life Sciences, University of the Chinese Academy of Sciences, Beijing, 10049, China
[e] Savaid Medical School, University of the Chinese Academy of Sciences, Beijing, 10049, China

ARTICLE INFO

Keywords:
Microfluidics
Lab-on-a-Chip
Dielectrophoresis
Bloodstream infection
Antimicrobial susceptibility testing

ABSTRACT

This paper describes an integrated microfluidic SlipChip device for rapid antimicrobial susceptibility testing (AST) of bloodstream pathogens in positive blood cultures. Unlike conventional AST methods, which rely on an overnight subculture of positive blood cultures to obtain isolated colonies, this device enables direct extraction and enrichment of the bacteria from positive blood cultures by dielectrophoresis. SlipChip technology enables parallel inoculation of the extracted bacteria into nanoliter-scale broth droplets to perform multiplexed ASTs simultaneously. The nanoliter confinement in the droplets increases the effective inoculation amount of the bacteria, shortens the diffusion distance of nutrient elements and gases, and allows faster growth and proliferation rates. Entropy-based image analysis used for the characterization of bacterial susceptibility patterns eliminates the requirement for single-cell morphological analysis and fluorescence labeling. As a proof-of-concept, the susceptibility patterns of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538p, and a positive blood culture containing *Escherichia coli* against several broad-spectrum antibiotics were determined by the SlipChip device. The on-chip AST results were well matched with those respectively reported by the broth microdilution method and a BD Phoenix Automated Microbiology System. Reliable AST results can be reported to clinicians within 3–8 h using this simple device after positive blood culture, allowing earlier proper administration of antimicrobial therapy.

1. Introduction

In this paper, we describe an integrated SlipChip device for rapid antimicrobial susceptibility testing (AST) of bloodstream pathogens in positive blood cultures without the requirement for the overnight subculture step. Bloodstream infection (BSI) is a life-threatening disease with high rates of morbidity and mortality. Sepsis, the most dangerous clinical manifestation of BSI, accounts for 60%–80% of infant mortality yearly (Kissoon et al., 2011; Pavlaki et al., 2013), and approximately 135,000 people in Europe and 215,000 in the United States die from sepsis annually (Tissari et al., 2010). Furthermore, BSI might even cause endocarditis, meningitis, and osteomyelitis infections through the hematogenous spread of bacteria, which further increases the rates of patient mortality (Corless et al., 2000; Lew and Waldvogel, 2004; Rothman et al., 2002).

In an era of global antibiotic resistance and absence of new antimicrobial drug development (Arnold et al., 2011; Laxminarayan et al., 2013; Leuthner and Doern, 2013), rapid AST of bloodstream pathogens is crucial since it provides clinicians with valuable information to determine appropriate and timely antimicrobial therapy, which improves clinical outcomes (Kumar et al., 2006). However, conventional AST methods widely used in clinical microbiology laboratories, including broth microdilution, disk diffusion, E-test, and automated systems, heavily rely on further overnight subculture after initial enrichment

* Corresponding author.State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China.
** Corresponding author.
*** Corresponding author.
E-mail addresses: xycpumch@139.com (Y.-C. Xu), lanying@im.ac.cn (Y. Lan), wenbin@im.ac.cn (W. Du).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bios.2019.04.003
Received 5 February 2019; Received in revised form 14 March 2019; Accepted 1 April 2019
Available online 04 April 2019
0956-5663/

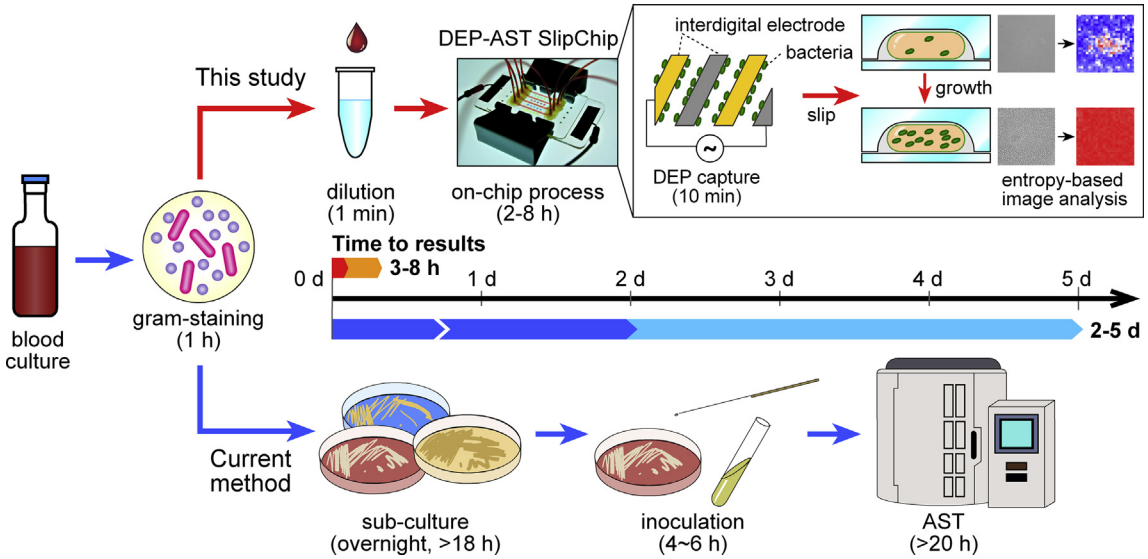


Fig. 1. Flowchart for AST of positive blood cultures using the SlipChip device and the automated system-based standard method. The on-chip AST process, including sample dilution, DEP capture, bacterial cultivation, and entropy-based image analysis steps, typically takes 3–8 h for reporting AST results. In comparison, the standard method, which includes the overnight subculture step, bacterial inoculation, and immunological/biochemical tests in the BD Phoenix automated microbiology system, typically takes 2–5 days for reporting AST results.

culture, which is laborious and time-consuming. As shown in Fig. 1, the whole process typically takes 1–3 days for a blood culture to become positive and an additional 2–4 days for reporting AST results (Carrigan et al., 2004).

To shorten the turnaround time for AST, several genotypic tests have been introduced (Mancini et al., 2010), e.g., LightCycler SeptiFast Test MGRADE (Roche Diagnostics, Risch-Rotkreuz, Switzerland), SepsiTest (Molzym Molecular Diagnostics, Bremen, Germany), and the IRIDICA BAC BSI assay (Abbott Diagnostics, Lake Forest, IL, USA). Nevertheless, these tests are typically more expensive than the conventional AST methods, are not applicable to the cases where newly acquired antibiotic resistance is independent of target gene expression (Kalashnikov et al., 2012), and cannot quantitatively obtain the bacterial susceptibility to particular antibiotics (Reller et al., 2009). Furthermore, genetically identical bacterial cells may exhibit different phenotypes (Balaban et al., 2004). Ultimately, the phenotypic AST method is still considered as the gold standard for the determination of antimicrobial susceptibility (Levy and Marshall, 2004; Mach et al., 2011).

An alternative method to shorten the turnaround time for AST is to inoculate an automated AST system directly from positive blood culture bottles without the overnight subculture step. Several studies have compared the direct and standard methods for different (combinations of) automated systems, which yielded reliable results for both Gram-positive and Gram-negative bacteria (Beuving et al., 2011; Bruins et al., 2004; Chapin and Musgnug, 2003; Funke and Funke-Kissling, 2004; Waites et al., 1998; Wellinghausen et al., 2007). The turnaround time required to report AST was highly dependent on the bacteria tested and the automated system used, which varied between 8 and 24 h after direct inoculation (Chapin and Musgnug, 2003; Funke and Funke-Kissling, 2004).

Recently, several microfluidic devices have been developed for phenotypic AST (Boedicker et al., 2008; Chen et al., 2010; Choi et al., 2014; Dong and Zhao, 2015; Lu et al., 2013; Sinn et al., 2011; Sun et al., 2016; Xu et al., 2016). The cultivation of bacteria in nanoliter-scale confinements increases the effective inoculation amount of the bacteria, shortens the diffusion distance of nutrient elements and gases, and allows released biochemical signals to accumulate in much smaller volumes, resulting in faster growth and proliferation rates (Boedicker et al., 2008; Chen et al., 2010). Furthermore, microfluidic devices enable the simultaneous implementation of numerous tests in the same experiment with lower reagent consumption, which is especially beneficial for rapid and accurate AST (Boedicker et al., 2008). Nevertheless, these microfluidic devices are mostly designed for purified subcultures, and the lack of on-chip sample preparation process still poses a significant challenge for their practical applications (Dong and Zhao, 2015). On the other hand, microscopic image analysis coupled with microfluidic devices enables the characterization of bacterial susceptibility patterns against several antibiotics at the single-cell level, which contributes to early AST (Choi et al., 2014; Lu et al., 2013; Mohan et al., 2013; Peitz and van Leeuwen, 2010). Nevertheless, high-resolution time-lapse microscopy and bacterial cell immobilization techniques are usually required, and the high diversity in microbial morphology, size, and motility makes the development of a universal image recognition algorithm difficult.

Previously, we developed a microfluidic device which integrated dielectrophoresis (DEP), SlipChip technology, and preloaded multiplex array PCR for rapid identification of bloodstream pathogens within 3 h (Cai et al., 2014). In that work, we proved that DEP could be used as a broad-spectrum capture method for various causative bacteria and fungi of BSIs; the multiplex array PCR was able to identify up to 20 bacterial species in one experiment. In this work, we demonstrate a DEP-AST SlipChip device, a new version of SlipChip developed for rapid phenotypic AST of bloodstream pathogens in positive blood cultures. The concept of image entropy was introduced to characterize the bacterial susceptibility patterns, which eliminates the requirement for single-cell morphology analysis and fluorescence labeling. As a proof-of-concept, the susceptibility patterns of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and a positive blood culture containing *E. coli* against several broad-spectrum antibiotics were determined by the SlipChip device, which were well matched with those respectively reported by the broth microdilution method and a BD Phoenix Automated Microbiology System. The whole on-chip AST process took 3–8 h after positive blood culture, making this method valuable for rapid antimicrobial administration and management in clinical.

2. Materials and methods

2.1. Preparation of samples

EDTA-treated blood samples were obtained from healthy donors at Peking Union Medical College Hospital (PUMCH, Beijing, China) and stored at 4 °C before use. The bacterial strains used in this study were *E. coli* ATCC 25922 carrying plasmid pACGFP1, *E. coli* RP437 carrying plasmid DsRedT.4 (Clontech Laboratories, Mountain View, CA, USA), and *S. aureus* ATCC 6538P without exogenous plasmid. Single colonies of these strains on cation-adjusted Mueller-Hinton broth (CAMHB) agar plates were separately inoculated into 5 mL of CAMHB for overnight culture at 37 °C. Aliquots of these bacterial solutions were centrifuged at 8000 g for 5 min. The obtained bacterial pellets were resuspended and adjusted using 100-fold diluted blood samples to yield a bacterial concentration of $\sim 1 \times 10^5$ CFU/mL for on-chip AST; or using CAMHB to yield a bacterial concentration of $\sim 1 \times 10^8$ CFU/mL (0.5 McFarland) for off-chip AST using the broth microdilution method on microtiter plates.

Clinical positive blood cultures were provided by the Department of Clinical Laboratory, PUMCH. The seeded blood culture bottle signaled positive in a BacT/ALERT 3D microbial detection system (bioMérieux, Inc., Durham, NC, USA) and appeared to be monomicrobial by Gram-staining was used in this study. An aliquot of the positive blood culture was directly used for on-chip AST after 100-fold dilution without subculture. In parallel to our on-chip AST, another aliquot of the positive blood culture was subjected to an overnight subculture from which a standard inoculum was prepared for introduction into a BD Phoenix Automated Microbiology System (Becton Dickinson and Company, Sparks, MD).

This study was approved by the Committee and Research Ethics Board of PUMCH. Informed consent was obtained from all blood donors and patients.

2.2. On-chip AST

On-chip ASTs were performed according to the Clinical & Laboratory Standards Institute (CLSI) protocol (CLSI, 2015) with minor adjustments. All antibiotics used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions (10 mg/mL) of ampicillin (AMP), moxifloxacin (MXF), cephalothin (CEP), and meropenem (MEM) were freshly prepared in sterile deionized water for on-chip ASTs of *E. coli* 25922 and *S. aureus* 6538p. The stock solutions (10 mg/mL) of cefotaxime (CTX), cefepime (FEP), meropenem (MEM), and levofloxacin (LEV) were freshly prepared in sterile deionized water for on-chip ASTs of the positive blood culture containing *E. coli*. A series of 10-fold dilutions of these stock solutions were prepared using CAMHB (0.01–100 μg/mL). 50 nL of each dilution series was preloaded into the microwells using the method described in the Supplementary Materials.

All on-chip experimental conditions used in this study are as described in the previous study (Cai et al., 2014). Briefly, 100-fold diluted blood samples were directly infused into the SlipChip device at a constant flow rate of 1 μL/min 10 mg/mL of Bovine Serum Albumin (Sigma-Aldrich, St. Louis, MO, USA) was added to the samples to avoid the adhesion of bacteria and blood cells to the sample channels. When a high-frequency alternating current (HFAC) signal of 20 Vpp (peak-to-peak voltage) and 20 MHz was applied between the interdigitated microelectrodes, the bacteria in the blood samples were extracted and enriched into the microgroove arrays. After DEP capture for 10 min, the device was slipped to overlay the microgrooves containing the captured bacteria on the bottom plate with the microwells preloaded with antibiotic-doped broth droplets on the top plate (Fig. 2A and B). As the HFAC signal between the interdigitated microelectrodes was removed, the captured bacteria were released and dispersed into the droplets. After slipping back the top plate, the device was covered by FC-70 oil to avoid evaporation. The on-chip bacterial cultivation at 37 °C was monitored using an Eclipse Ti inverted microscope (Nikon, Japan) equipped with a humidified microscope incubator (World Precision Instruments, USA). Two objectives, Plan Fluor 10 × /0.3 Ph1 and Super Plan Fluor 20x/0.45 Ph1, were used under phase contrast settings to image the on-chip bacterial cultivation every half an hour. The obtained images were characterized using the entropy-based image analysis approach to determine bacterial susceptibility patterns. The on-chip ASTs for each bacterial strain were repeated for 5 times. More details regarding device configuration, fabrication, assembly and off-chip AST are provided in the Supplementary Materials.

2.3. Entropy-based image analysis

The bacterial antimicrobial susceptibility was determined using the entropy-based image analysis approach. The captured bright-field images were divided into matrices of 20 × 20-pixel segments (Fig. 3A), which are analogous to the size of a few bacterial cells. The entropy value H_{xy} of each segment was calculated using equation (1):

$$H_{xy} = -\sum_{i=0}^{255} P_i \log_2 P_i \tag{1}$$

where

$$P_i = f_i / N^2 \tag{2}$$

here, i ($0 \le i \le 255$) represents the gray value of the pixel, P_i represents the proportion of the gray value in this segment as i pixels, f_i is the frequency of i, and N^2 is the scale of the segment ($N = 20$). The entropy values of all segments were calculated to obtain an entropy matrix H. Min-max normalization was used to eliminate the discrepancies caused by bacterial species or the microenvironment using equation (3)

$$H'_{xy} = \frac{H_{xy} - \min(H)}{\max(H) - \min(H)} \tag{3}$$

where $x = (x_1, \ldots, x_n)$, $y = (y_1, \ldots, y_n)$, and H'_{xy} is the normalized data ($n = 25$). The normalized results were presented in a heatmap manner and sorted in descending order for better direct-viewing and comparison.

3. Results and discussion

3.1. Design of the SlipChip device for direct AST

To simplify and expedite the AST process, we designed the DEP-AST SlipChip device, which incorporates DEP, SlipChip technology, microdroplet array, and microscopic image analysis into an integrated and portable system. Unlike conventional AST methods, which heavily rely on an overnight subculture of positive blood cultures to obtain isolated colonies, this device employs DEP for direct extraction and enrichment of the bacteria from positive blood cultures. SlipChip technology enables parallel inoculation of the extracted bacteria into nanoliter-scale antibiotic-doped broth droplets to perform multiple ASTs simultaneously. The confinement in nanoliter droplets increases the effective inoculation amount of the bacteria, shortens the diffusion distance of nutrient elements and gases, and allows released biochemical signals to accumulate in much smaller volumes, resulting in faster growth and proliferation rates. The entropy-based image analysis approach used for the characterization of bacterial susceptibility patterns eliminates the requirement for single-cell morphological analysis and fluorescence labeling.

The DEP-AST SlipChip device was operated according to the following steps (Fig. 2B): *i*) broth droplets with or without antibiotics were first preloaded into the microwells, and a 100-fold diluted positive blood culture was directly infused into the device at a flow rate of 1 μL/min; *ii*) an HFAC signal of 20 Vpp and 20 MHz was applied between the

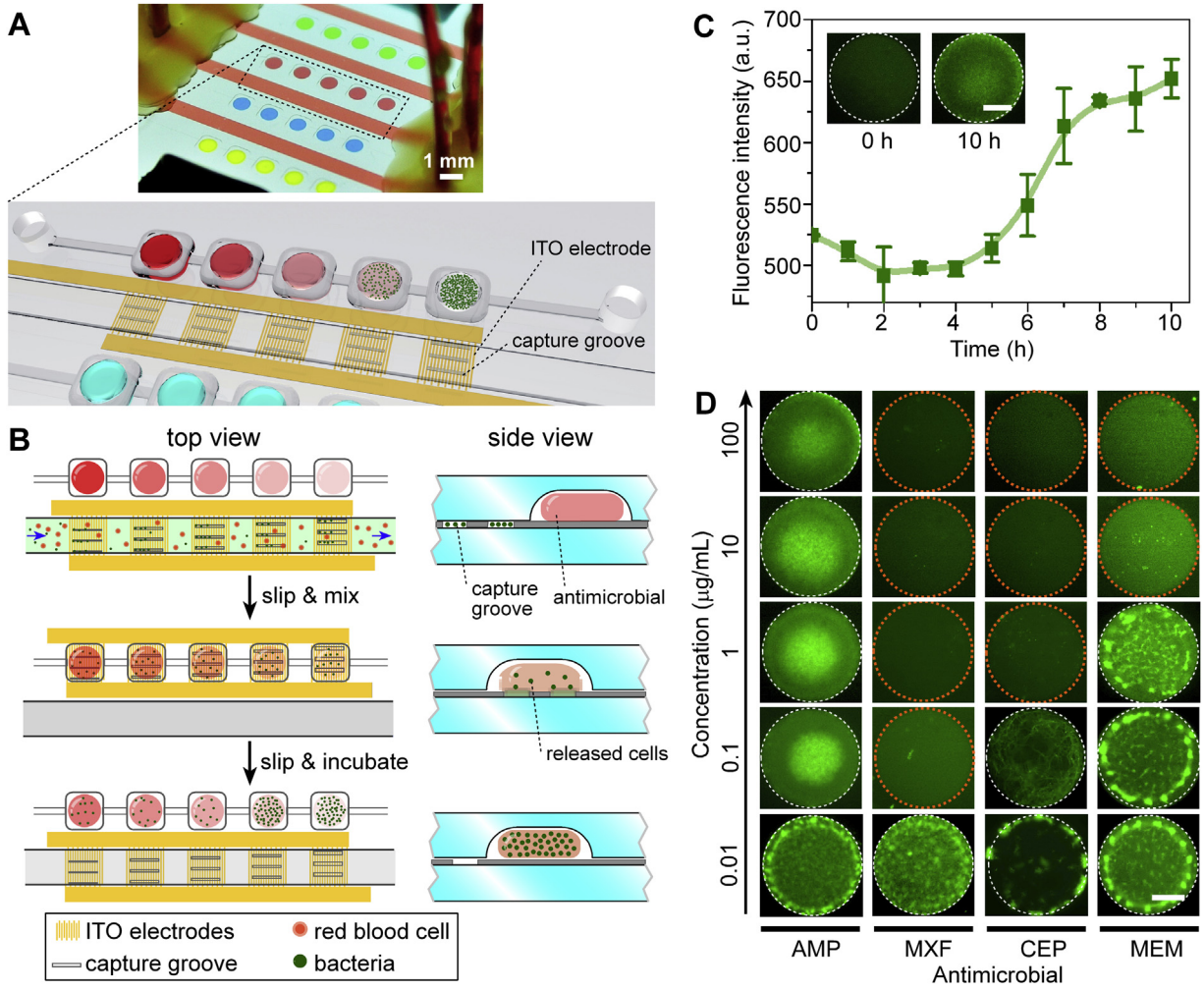


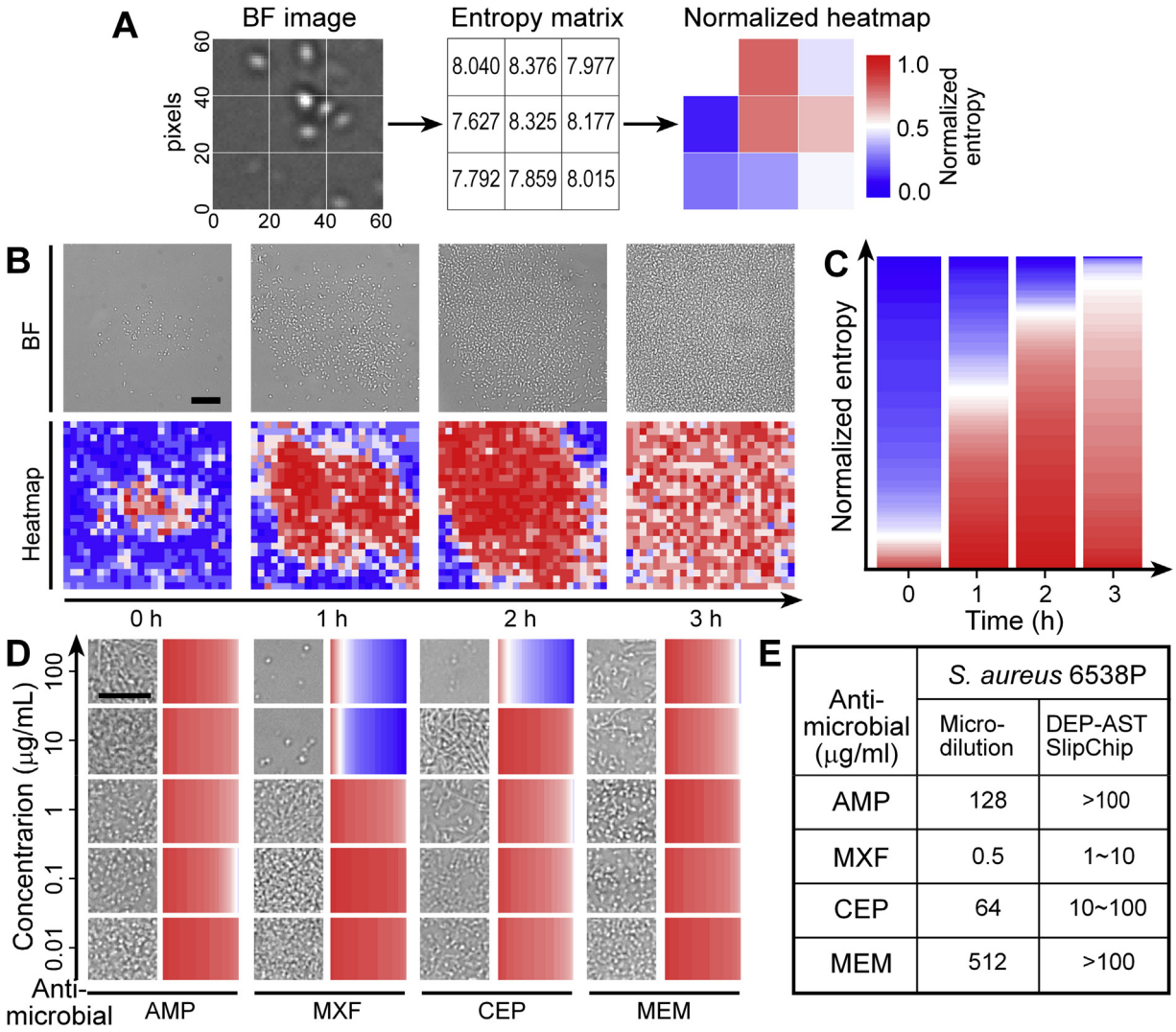
Fig. 2. Applicability of the SlipChip device for bacterial cultivation and ASTs. A) Image of the SlipChip device preloaded with food dye solutions in the microwells; B) Schematic diagram of device operation in top and side views; C) Fluorescence images of on-chip cultivation of GFP-labeled *E. coli* 25922 in pure broth droplets at time points of 0 and 10 h, and the growth curve originating from the fluorescence intensity of the droplets; D) Fluorescence images of GFP-labeled *E. coli* cultivated in the broth droplets doped with AMP, MXF, CEP, and MEM for 10 h. Scale bars are 200 μm.

interdigitated microelectrodes; *iii*) after DEP capture for 10 min, the top plate was slipped to overlay the microgrooves containing the bacteria (green dots) with the microwells preloaded with antibiotic-doped broth droplets, and the signal applied between the microelectrodes was removed to release the bacteria into the droplets; *iv*) finally, the top plate was slipped back to its original position to evade the contaminated surface, and then the device was incubated at 37 °C for microscopic imaging.

Compared to the automated system-based standard method which takes 2–4 days for reporting AST results after positive blood culture (Fig. 1), this integrated DEP-AST SlipChip device enables direct AST of bloodstream pathogens without the requirement for the overnight subculture step, shortening the time-to-result to approximately 3–8 h. Furthermore, the proposed entropy-based image analysis approach excludes the effects of morphological variation in bacterial size, shape, motility, and growth rate on image analysis, which may cause increased complexity in automated analysis of real-world samples. Also, this SlipChip device was specifically designed for monomicrobial infections, which account for approximately 90% of BSIs (Carrigan et al., 2004; Lee et al., 2007; Pavlaki et al., 2013).

3.2. Applicability of the SlipChip device for bacterial cultivation and direct AST

Bacterial cultivation on the SlipChip device without adverse effects is a prerequisite for rapid and accurate AST. A benchmark strain of GFP-labeled *E. coli* 25922 was used to evaluate the effects of the microenvironment provided by the device on bacterial cultivation. It has been reported that DEP treatment of bacteria at 20 Vpp and 15 MHz for 1 h did not cause any change in cell growth, and the damage in cells is related to the duration of DEP treatment instead of the frequency of applied voltage (Yang et al., 2008). Therefore, the used DEP treatment at 20 Vpp and 20 MHz for 10 min would not affect the following cell growth and proliferation procedures. Since the bacteria were suspended in diluted blood samples, the hypotonic effect on bacterial viability was evaluated by spiking GFP-labeled *E. coli* 25922 in 100-fold diluted blood samples for 20 min. It was found that most of the bacteria (91.7% ± 6.2) were still viable and cultivatable. The bacterial cultivation in the pure CAMHB droplets at time points of 0 and 10 h is shown in Fig. 2C. It was found that the fluorescence intensity of the droplets gradually increased over time, indicating the growth and proliferation of the bacteria. The fluorescence intensity of the droplets (n = 5) was plotted *versus* time to derive a growth curve. The curve could be fitted with a sigmoidal growth model and showed a similar shape compared to that obtained from bulk cultivation, both indicating



Anti-microbial (μg/ml)	*S. aureus* 6538P	
	Micro-dilution	DEP-AST SlipChip
AMP	128	>100
MXF	0.5	1~10
CEP	64	10~100
MEM	512	>100

Fig. 3. Characterization of bacterial cultivation and susceptibility patterns using the entropy-based image analysis approach. A) Image analysis process; B) Bright-field images of on-chip cultivation of the unlabeled *S. aureus* 6538p in pure broth droplets at time points of 0, 1, 2, and 3 h with the corresponding entropy heatmaps; C) Heat plots in a descending order converted from the entropy heatmaps; D) Bright-field images of the unlabeled *S. aureus* 6538p cultivated in the broth droplets doped with AMP, MXF, CEP, and MEM for 2 h and the corresponding entropy heatmaps; E) AST results reported by the SlipChip device and the broth microdilution method. Scale bars are 50 μm.

the appropriate microenvironment provided by the device for bacterial cultivation.

The benchmark strain of *E. coli* 25922 was further used to evaluate the applicability of the device for direct AST. After DEP capture, the bacteria were parallel inoculated into the broth droplets doped with antibiotics of AMP, MXF, CEP, and MEM. The bacterial concentration in the 50-nL droplets was ~1.9×10^6 CFU/mL (94.1% capture efficiency according to the previous study), which was almost consistent to the bacterial concentration (~2×10^6 CFU/mL) used in the broth microdilution experiments. The fluorescence images of bacterial cultivation for 10 h in the antibiotic-doped broth droplets are shown in Fig. 2D. The growth and proliferation of GFP-labeled *E. coli* 25922, which had acquired the resistance to AMP by the transfection of plasmid pACGFP1, were observed in the presence of all concentrations of AMP tested. Nevertheless, the growth and proliferation of *E. coli* 25922 were completely inhibited in the presence of 0.1 μg/mL MXF, 1 μg/mL CEP, and 10 μg/mL MEM. The on-chip AST results were well matched with those reported by the broth microdilution experiments performed on microtiter plates (Fig. 2D and Table S1), indicating the applicability of the device for direct AST.

3.3. Entropy-based analysis of bacterial cultivation and susceptibility patterns

In image processing, entropy is defined as corresponding states of intensity level that individual pixels can adapt (Hinojosa et al., 2018; Monti et al., 2009; Shiozaki, 1986). It is used in the quantitative analysis and evaluation of image disorders, as it provides a detailed comparison of image textures. In this study, the on-chip bacterial cultivation visualized by bright-field images was characterized using the entropy-based image analysis approach, since bacterial growth and proliferation can lead to a change in image textures. By dividing the image into a matrix of small rectangular segments and calculating the entropy of each segment, we could analyze the dynamic growth and proliferation of bacterial cells, as well as their spatial distribution.

A blood sample spiked with a gram-positive strain of *S. aureus* 6538P was used to validate the applicability of the entropy-based image analysis approach. After DEP capture and parallel inoculation into the pure broth droplets, the bright-field images of bacterial cultivation at time points of 0, 1, 2, and 3 h are shown in Fig. 3B. Using the image entropy analysis process shown in Fig. 3A, the corresponding entropy values in a heatmap manner could be obtained, which were used as a

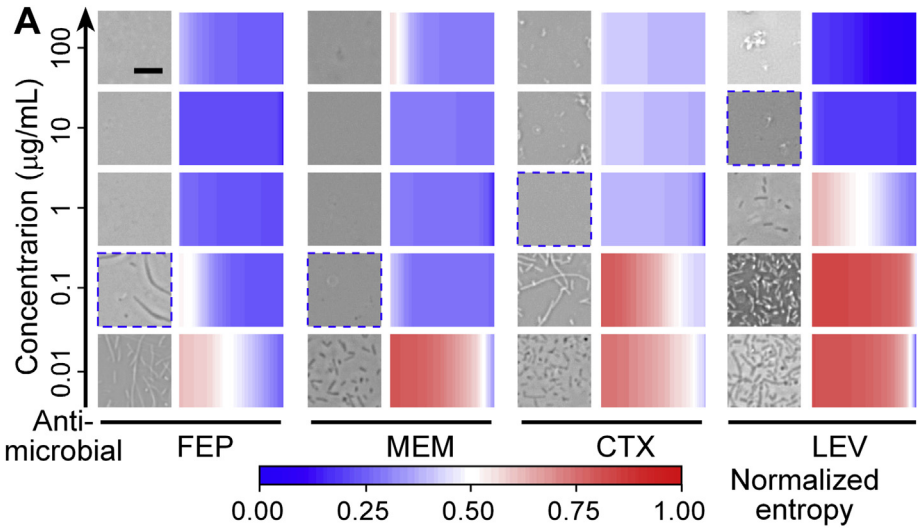


Fig. 4. Rapid AST of a positive blood culture using the SlipChip device. A) Bright-field images of the clinical isolate of *E. coli* cultivated in the broth droplets doped with FEP, MEM, CTX, and LEV for 2 h and the corresponding entropy heatmaps; B) The AST results reported by the SlipChip device and the automated system-based standard method. Scale bar is 25 μm.

B

Anti-microbial (μg/ml)	*E. coli* 14B09732	
	BD phoenix	DEP-AST SlipChip
FEP	<=2	0.01~0.1
MEM	<=1	0.01~0.1
CTX	<=1	0.1~1
LEV	>8	1~10

parameter to determine the bacterial growth and proliferation and their spatial distribution in this viewing field. The entropy heatmaps were converted into heat plots in a descending order to determine the occupied space by bacterial cells (Fig. 3C). Significantly, this entropy-based image analysis approach was sensitive enough to indicate the bacterial growth and proliferation within 2 h after bacterial inoculation (Fig. 3B and C). Furthermore, the on-chip cultivation of a motile Gram-negative strain, RFP-labeled *E. coli*, was determined by the entropy-based image analysis approach, which yielded a quite comparable growth curve to that obtained from the fluorescence-based image analysis approach. Importantly, the entropy-based image analysis approach characterized the on-chip bacterial cultivation approximately 4 h earlier than the fluorescence-based image analysis approach (Fig. S1).

Next, the entropy-based image analysis approach was further used to determine the susceptibility patterns of *S. aureus* 6538P against AMP, MXF, CEP, and MEM. As mentioned above, the bacterial concentration in the 50-nL antibiotic-doped droplets was almost consistent with the bacterial concentration used in the broth microdilution experiments. The bright-field images of bacterial cultivation for 2 h and the corresponding entropy heatmaps are shown in Fig. 3D. The entropy heatmaps indicated the growth and proliferation of *S. aureus* 6538p in the presence of all concentrations of AMP and MEM tested, and complete inhibition of growth and proliferation in the presence of 10 μg/mL MXF and 100 μg/mL CEP. The on-chip AST results were closely matched with those reported by the broth microdilution method (Fig. 3E), indicating the applicability of the entropy-based image analysis approach for the determination of bacterial susceptibility patterns. Compared to the microscopic image analysis methods reported in previous studies, the proposed approach in this work allows characterizing the spatial distribution of bacteria within the viewing field without the requirement for complicated readouts or rigorous image acquisition setups.

3.4. Rapid AST of a positive blood culture

To demonstrate the applicability of the DEP-AST SlipChip device in practical situations, we determined antimicrobial susceptibility of a positive blood culture using the device. An aliquot of the positive blood culture was directly infused into the device after 100-fold dilution. After DEP capture, the bacteria were parallel inoculated into the broth droplets doped with FEP, MEM, CTX, and LEV. The bright-field images of bacteria cultivated for 2 h and the entropy values of the droplet centers are shown in Fig. 4. The bacterial growth and proliferation were completely inhibited in the presence of 1 μg/mL CTX, 0.1 μg/mL FEP, and 0.1 μg/mL MEM, indicating the susceptibility of this clinical isolate to these antibiotics. The inhibitory concentration of LEV was 10 μg/mL, indicating that LEV might be ineffective in the treatment of this bacterial infection. The on-chip AST results were closely matched with those reported by the automated system-based standard method (Fig. 4B). According to the record of PUMCH, it took approximately 18 h for subculture and another 10 h for ASTs. The on-chip AST results of the clinical isolate were available within 3 h after positive blood culture, which was 1 day earlier than the automated system-based standard method.

4. Conclusion

In this work, a DEP-AST SlipChip device which integrates DEP, SlipChip technology, microdroplet array, and microscopic image analysis was developed for direct AST of positive blood cultures. DEP, serving as an on-chip sample preparation method, enables direct extraction and enrichment of the bacteria from positive blood cultures. Meanwhile, it eliminates the interference of the following image analysis process caused by blood cells. SlipChip technology enables parallel inoculation of the extracted bacteria into a broth droplet array to perform multiple ASTs simultaneously. The confinement in nanoliter droplets increases the effective inoculation amount of the bacteria,

shortens the diffusion distance of nutrient elements and gases, and allows released biochemical signals to accumulate in much smaller volumes, resulting in faster growth and proliferation rates. The proposed entropy-based image analysis approach used for the characterization of bacterial cultivation eliminates the requirement for single-cell morphological analysis and fluorescence labeling. Furthermore, this approach provides a direct view of AST results without the requirement for high-resolution imaging. As a proof-of-concept, the susceptibility patterns of *E. coli* ATCC 25922, *S. aureus* ATCC 6538p, and a positive blood culture containing *E. coli* against several broad-spectrum antibiotics were determined by the device, which were well matched with those reported by the standard methods.

Future work will be directed toward expanding the scale of the droplet array on one device to screen more antibiotics and dilution series, validating the entropy-based analysis approach for various bacterial and fungal species, and performing a large-scale side-by-side comparison in the performance of the DEP-AST SlipChip device and current gold-standard methods to evaluate the clinical statistical significance of our methods. Efforts will also be directed toward integrating the DEP-AST SlipChip device with on-chip isothermal nucleic acid amplification, such as recombinase polymerase amplification (RPA), for simultaneous identification and AST of bloodstream pathogens.

Declaration of interests

None.

CRediT authorship contribution statement

Qiaolian Yi: Investigation, Data curation, Writing - original draft, Writing - review & editing. **Dongyang Cai:** Investigation, Data curation, Writing - original draft, Writing - review & editing. **Meng Xiao:** Investigation, Data curation, Writing - original draft, Writing - review & editing. **Mengyue Nie:** Writing - review & editing. **Qinna Cui:** Methodology, Resources. **Jingwei Cheng:** Methodology, Resources. **Caiming Li:** Visualization. **Jie Feng:** Supervision, Resources. **Gerald Urban:** Supervision, Resources. **Ying-Chun Xu:** Conceptualization, Supervision, Formal analysis, Writing - original draft, Writing - review & editing. **Ying Lan:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. **Wenbin Du:** Conceptualization, Supervision, Formal analysis, Writing - original draft, Writing - review & editing.

Acknowledgments

This study was supported by the Ministry of Science and Technology of the People´s Republic of China (2016YFC0100904, 2016YFE0205800), the National Natural Science Foundation of China (31470221, 21822408), the Beijing Municipal Science and Technology Commission (Z161100000116042), the Key Research Program of the Chinese Academy of Sciences (KFZD-SW-219-4), and the Key Program of Frontier Sciences of Chinese Academy of Sciences (QYZDB-SSW-SMC008).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.04.003.

References

Arnold, H., Micek, S., Skrupky, L., Kollef, M., 2011. Antibiotic stewardship in the intensive care unit. Semin. Resp. Crit. Care 32 (02), 215–227.
Balaban, N.Q., Merrin, J., Chait, R., Kowalik, L., Leibler, S., 2004. Bacterial persistence as a phenotypic switch. Science 305 (5690), 1622–1625.
Beuving, J., van der Donk, C.F., Linssen, C.F., Wolffs, P.F., Verbon, A., 2011. Evaluation of direct inoculation of the BD PHOENIX system from positive BACTEC blood cultures for both Gram-positive cocci and Gram-negative rods. BMC Microbiol. 11, 156.
Boedicker, J.Q., Li, L., Kline, T.R., Ismagilov, R.F., 2008. Detecting bacteria and determining their susceptibility to antibiotics by stochastic confinement in nanoliter droplets using plug-based microfluidics. Lab. Chip 8 (8), 1265–1272.
Bruins, M.J., Bloembergen, P., Ruijs, G.J.H.M., Wolfhagen, M.J.H.M., 2004. Identification and susceptibility testing of enterobacteriaceae and Pseudomonas aeruginosa by direct inoculation from positive BACTEC blood culture bottles into vitek 2. J. Clin. Microbiol. 42 (1), 7–11.
Cai, D., Xiao, M., Xu, P., Xu, Y., Du, W., 2014. An integrated microfluidic device utilizing dielectrophoresis and multiplex array PCR for point-of-care detection of pathogens. Lab. Chip 14 (20), 3917–3924.
Carrigan, S.D., Scott, G., Tabrizian, M., 2004. Toward resolving the challenges of sepsis diagnosis. Clin. Chem. 50 (8), 1301–1314.
Chapin, K.C., Musgnug, M.C., 2003. Direct susceptibility testing of positive blood cultures by using sensititre broth microdilution plates. J. Clin. Microbiol. 41 (10), 4751–4754.
Chen, C.H., Lu, Y., Sin, M.L.Y., Mach, K.E., Zhang, D.D., Gau, V., Liao, J.C., Wong, P.K., 2010. Antimicrobial susceptibility testing using high surface-to-volume ratio microchannels. Anal. Chem. 82 (3), 1012–1019.
Choi, J., Yoo, J., Lee, M., Kim, E., Lee, J.S., Lee, S., Joo, S., Song, S.H., Kim, E., Lee, J.C., Kim, H.C., Jung, Y., Kwon, S., 2014. A rapid antimicrobial susceptibility test based on single-cell morphological analysis. Sci. Transl. Med. 6 (267) 267ra174.
CLSI, 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement, Twenty-Fifth Informational Supplemented. Clinical & Laboratory Standards Institute, Wayne, PA.
Corless, C.E., Guiver, M., Borrow, R., Edwards-Jones, V., Fox, A.J., Kaczmarski, E.B., 2000. Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. J. Clin. Microbiol. 39 (4), 1553–1558.
Dong, T., Zhao, X., 2015. Rapid identification and susceptibility testing of uropathogenic microbes via immunosorbent ATP-bioluminescence assay on a microfluidic simulator for antibiotic therapy. Anal. Chem. 87 (4), 2410–2418.
Funke, G., Funke-Kissling, P., 2004. Use of the BD PHOENIX automated microbiology system for direct identification and susceptibility testing of gram-negative rods from positive blood cultures in a three-phase trial. J. Clin. Microbiol. 42 (4), 1466–1470.
Hinojosa, S., Dhal, K.G., Elaziz, M.A., Oliva, D., Cuevas, E., 2018. Entropy-based imagery segmentation for breast histology using the Stochastic Fractal Search. Neurocomputing 321, 201–215.
Kalashnikov, M., Lee, J.C., Campbell, J., Sharon, A., Sauer-Budge, A.F., 2012. A microfluidic platform for rapid, stress-induced antibiotic susceptibility testing of Staphylococcus aureus. Lab Chip 12 (21), 4523–4532.
Kissoon, N., Carcillo, J.A., Espinosa, V., Argent, A., Devictor, D., Madden, M., Singhi, S., van der Voort, E., Latour, J., 2011. World federation of pediatric intensive care and critical care societies: global sepsis initiative*. Pediatr. Crit. Care ME 12 (5), 494–503.
Kumar, A., Roberts, D., Wood, K.E., Light, B., Parrillo, J.E., Sharma, S., Suppes, R., Feinstein, D., Zanotti, S., Taiberg, L., Gurka, D., Kumar, A., Cheang, M., 2006. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock*. Crit. Care Med. 34 (6), 1589–1596.
Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K., Wertheim, H.F., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., Greko, C., So, A.D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A.Q., Qamar, F.N., Mir, F., Kariuki, S., Bhutta, Z.A., Coates, A., Bergstrom, R., Wright, G.D., Brown, E.D., Cars, O., 2013. Antibiotic resistance-the need for global solutions. Lancet Infect. Dis. 13 (12), 1057–1098.
Lee, A., Mirrett, S., Reller, L.B., Weinstein, M.P., 2007. Detection of bloodstream infections in adults: how many blood cultures are needed? J. Clin. Microbiol. 45 (11), 3546–3548.
Leuthner, K.D., Doern, G.V., 2013. Antimicrobial stewardship programs. J. Clin. Microbiol. 51 (12), 3916–3920.
Levy, S.B., Marshall, B., 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10, S122–S129.
Lew, D.P., Waldvogel, F.A., 2004. Osteomyelitis. Lancet (N. Am. Ed.) 364 (9431), 369–379.
Lu, Y., Gao, J., Zhang, D.D., Gau, V., Liao, J.C., Wong, P.K., 2013. Single cell antimicrobial susceptibility testing by confined microchannels and electrokinetic loading. Anal. Chem. 85 (8), 3971–3976.
Mach, K.E., Wong, P.K., Liao, J.C., 2011. Biosensor diagnosis of urinary tract infections: a path to better treatment? Trends Pharmacol. Sci. 32 (6), 330–336.
Mancini, N., Carletti, S., Ghidoli, N., Cichero, P., Burioni, R., Clementi, M., 2010. The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin. Microbiol. Rev. 23 (1), 235–251.
Mohan, R., Mukherjee, A., Sevgen, S.E., Sanpitakseree, C., Lee, J., Schroeder, C.M., Kenis, P.J.A., 2013. A multiplexed microfluidic platform for rapid antibiotic susceptibility testing. Biosens. Bioelectron. 49, 118–125.
Monti, D., Ariano, P., Distasi, C., Zamburlin, P., Bernascone, S., Ferraro, M., 2009. Entropy measures of cellular aggregation. Physica A 388 (13), 2762–2770.
Pavlaki, M., Poulakou, G., Drimousis, P., Adamis, G., Apostolidou, E., Gatselis, N.K., Kritselis, I., Mega, A., Mylona, V., Papatsoris, A., Pappas, A., Prekates, A., Raftogiannis, M., Rigaki, K., Sereti, K., Sinapidis, D., Tsangaris, I., Tzanetakou, V., Veldekis, D., Mandragos, K., Giamarellou, H., Dimopoulos, G., 2013. Polymicrobial bloodstream infections: epidemiology and impact on mortality. J. Glob. Antimicrob. Re. 1 (4), 207–212.
Peitz, I., van Leeuwen, R., 2010. Single-cell bacteria growth monitoring by automated

DEP-facilitated image analysis. Lab. Chip 10 (21), 2944.
Reller, L.B., Weinstein, M., Jorgensen, J.H., Ferraro, M.J., 2009. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin. Infect. Dis. 49 (11), 1749–1755.
Rothman, R.E., Majmudar, M.D., Kelen, G.D., Madico, G., Gaydos, C.A., Walker, T., Quinn, T.C., 2002. Detection of bacteremia in emergency department patients at risk for infective endocarditis using universal 16S rRNA primers in a decontaminated polymerase chain reaction assay. J. Infect. Dis. 186 (11), 1677–1681.
Shiozaki, A., 1986. Edge extraction using entropy operator. Comput. Vision Graph. Image Process 36 (1), 1–9.
Sinn, I., Kinnunen, P., Albertson, T., McNaughton, B.H., Newton, D.W., Burns, M.A., Kopelman, R., 2011. Asynchronous magnetic bead rotation (AMBR) biosensor in microfluidic droplets for rapid bacterial growth and susceptibility measurements. Lab. Chip 11 (15), 2604–2611.
Sun, H., Liu, Z., Hu, C., Ren, K., 2016. Cell-on-hydrogel platform made of agar and alginate for rapid, low-cost, multidimensional test of antimicrobial susceptibility. Lab. Chip 16 (16), 3130–3138.
Tissari, P., Zumla, A., Tarkka, E., Mero, S., Savolainen, L., Vaara, M., Aittakorpi, A., Laakso, S., Lindfors, M., Piiparinen, H., Mäki, M., Carder, C., Huggett, J., Gant, V., 2010. Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. Lancet (N. Am. Ed.) 375 (9710), 224–230.
Waites, K.B., Brookings, E.S., Moser, S.A., Zimmer, B.L., 1998. Direct susceptibility testing with positive BacT/Alert blood cultures by using MicroScan overnight and rapid panels. J. Clin. Microbiol. 36 (7), 2052–2056.
Wellinghausen, N., Pietzcker, T., Poppert, S., Belak, S., Fieser, N., Bartel, M., Essig, A., 2007. Evaluation of the merlin MICRONAUT system for rapid direct susceptibility testing of gram-positive cocci and gram-negative bacilli from positive blood cultures. J. Clin. Microbiol. 45 (3), 789–795.
Xu, B., Du, Y., Lin, J., Qi, M., Shu, B., Wen, X., Liang, G., Chen, B., Liu, D., 2016. Simultaneous identification and antimicrobial susceptibility testing of multiple uropathogens on a microfluidic chip with paper-supported cell culture arrays. Anal. Chem. 88 (23), 11593–11600.
Yang, L., Banada, P.P., Bhunia, A.K., Bashir, R., 2008. Effects of dielectrophoresis on growth, viability and immuno-reactivity of Listeria monocytogenes. J. Biol. Eng. 2 (1), 6.

DOI: 10.1016/j.bios.XXXX.XX.XXX

Supplementary Materials

Direct Antimicrobial Susceptibility Testing of Bloodstream Infection on SlipChip

Qiaolian Yi[a, d, #], Dongyang Cai[a, c, #], Meng Xiao[b], Mengyue Nie[a, d], Qinna Cui[a, d], Jingwei Cheng[b], Caiming Li[a, d], Jie Feng[a, d], Gerald Urban[c], Ying-Chun Xu[b] *, Ying Lan[a] *, and Wenbin Du[a, d, e] *

[a] State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

[b] Department of Clinical Laboratory, Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100101, China

[c] Department of Microsystems Engineering (IMTEK), University of Freiburg, Freiburg 79110, Germany

[d] College of Life Sciences, University of the Chinese Academy of Sciences, Beijing 10049, China

[e] Savaid Medical School, University of the Chinese Academy of Sciences, Beijing 10049, China

[#] These authors contribute equally to this work.

* Corresponding authors:
Ying-Chun Xu, E-mail address: xycpumch@139.com, Tel.: +86 010 69159766
Ying Lan, E-mail address: lanying@im.ac.cn, Tel.: +86 010 82994195
Wenbin Du, E-mail address: wenbin@im.ac.cn, Tel.: +86 010 82994195

Content:

Pages: 4

Figures: 1

Tables: 1

Materials and Methods

1. Fabrication and assembly of the SlipChip device

The device was fabricated based on the standard photolithography and wet etching techniques. The top plate (2.5 cm × 3 cm) containing four sample channels (800 μm in width, 15 μm in depth) and four air passages (100 μm in width, 5 μm in depth) was made of 0.7 mm thick Photoresist Coated Chromium-on-Glass substrate (Telic Co., Valencia, CA, USA). Five microwells (800 μm × 800 μm in size, 135 μm in depth) for preloaded antibiotic-doped broth droplets spaced at a regular interval on one side of each sample channel. The bottom plate (2.5 cm × 4.5 cm) was made of 1 mm thick ITO glass with interdigitated ITO microelectrode (900 μm in length, 35 μm in width, and 25 μm in space) arrays. The microgrooves (40 μm to 67 μm in width, 600 μm in length, and 5 μm in depth) were fabricated by photolithography of a 5 μm thick SU-8 2005 coating on the bottom plate. Both the top and bottom plate were silanized with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma-Aldrich, St. Louis, MO, USA) after plasma treatment to make the surface hydrophobic prior to use.

2. Off-chip ASTs using the microdilution method and an automated system

Off-chip ASTs using the broth microdilution method were performed according to the Clinical & Laboratory Standards Institute (CLSI) protocol. Briefly, antimicrobial stock solutions (10 mg/mL) of AMP, MXF, CEF, and MEM were freshly prepared in sterile deionized water. A series of 10-fold dilution of the stock solutions were prepared using CAMHB. Appropriate volumes of these dilutions were added to CAMHB to obtain 2-fold dilution series on microtiter plates with a volume of 200 μL. Bacterial solutions (~1×10^8 CFU/mL, 0.5 McFarland) of 4 μL volumes were added to the 2-fold dilution series to yield a final bacterial concentration of ~2×10^6 CFU/mL in each well. The microtiter plates were incubated at 37 ºC for 24 hours and the optical density (OD600) of each well was detected by a microplate reader. The off-chip ASTs using the broth microdilution method were repeated for 3 times.

An aliquot of the positive blood culture was Gram-stained and inoculated onto a blood agar plate which was then incubated overnight at 37 ºC to obtain isolate colonies. A standardized bacterial solution (0.5 McFarland) was prepared and then inoculated into the appropriate panel of the BD Phoenix automated identification and susceptibility testing system. The AST results were obtained following the manufacturer's instructions.

Supplementary Figures and Tables.

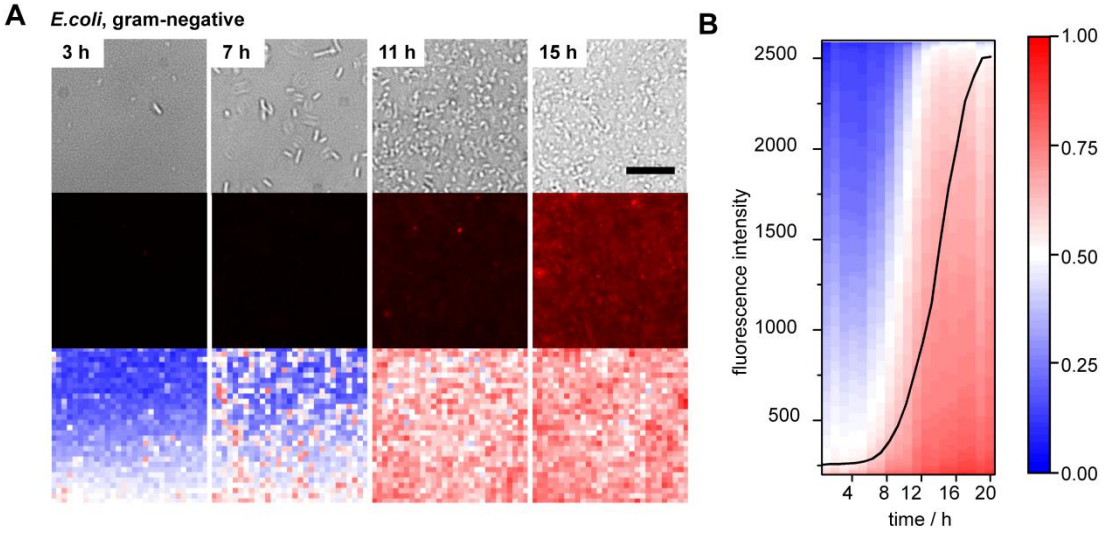


Figure S1. Side-by-side comparison of fluorescence-based and entropy-based image analysis methods for characterizing the growth and proliferation of *E. coli* RP437 carrying plasmid DsRedT.4 on the DEP-AST SlipChip. A) The time-lapse bright-field images, the fluorescence images, and the corresponding entropy heatmaps of RFP-labeled *E. coli* RP437 cultivated on the device; B) Heat plots in a descending order converted from the entropy heatmaps; the fluorescence intensity of the droplets was plotted *versus* time to derive a growth curve. Scale bar is 100 μm.

Table S1. Comparison of the AST results reported by the DEP-AST SlipChip device and the broth microdilution method.

Name	*E.coli* 25922 carrying plasmid pACGFP1	
	Normal method	Chip method
Ampicillin	>100	>100
Moxifloxacin	0.016	0.01~0.1
Cephalothin	0.25	0.1~1
Meropenem	4	1~10