New automation techniques in diagnostic microbiology

Presented by

M. Hakemi-Vala

Associate Prof. SBMU

Introduction

- Conventional approaches to bacterial **ID** and **AST** typically rely on **culture-based** approaches that take 2 to 7 days to return results. The long turnaround times contribute to **the spread of infectious disease, negative patient outcomes, and the misuse of antibiotics that can contribute to antibiotic resistance.**
- Also, to identify organisms difficult to culture or newly emerging strains, are other limitations of conventional methods(CM).

Cont'd

- In addition , by global emergence of multi drug resistant (MDR) infectious agents now days;
- **Need to rapid diagnostic methods** with reproducibility, high specificity and sensitivity are in increasing.
- In comparison to chemistry specimens, microbiology specimens are much more complex.
- Furthermore, by manually processing of samples, incubation times and processing itself are not guaranteed **standardized and qualitatively equivalent.**
- So, we are going to introduce new solutions, in continue.

Solutions

- It is the development of molecular-level assays that employ enzymatic amplification of pathogen nucleic acids. Such as:, **PCR**, **fluorescent *in situ* hybridization (PNA-FISH)** and **targeted real-time PCR** such as GeneXpert assays.
- **Advantage:**
- Means to **rapid diagnosis**
- **Disadvantages:**

✓ Molecular analysis faces limitations when **broad resistance profiles** must be surveyed (such as; different ESBLs genes).

✓ Uncharacterized resistance genes are **not amenable to molecular level testing.**

Cont'd

- Therefore, it is of interest to develop **next-generation technologies** that speed the direct phenotypic testing of bacteria and monitor the effects of antimicrobial therapies.
- However, automation was introduced many years ago for many diagnostic disciplines for instance in chemistry and hematology, the first automation system for clinical bacteriology was released in 2006, and it rapidly proved its value by increasing productivity, allowing a continuous increase in sample volumes despite limited budgets and personnel shortages.

Cont'd

- Recently, total laboratory automation (TLA) systems have been developed by several companies. here are **three TLA solutions** ; available, Kiestra TLA (BD Kiestra B.V., Drachten, Netherlands), full microbiology laboratory automation (FMLA; bio Mérieux, Inc., La Balme, France), and the WASPLab (Copan Diagnostics, Murrieta, CA).
- They all include track systems to move plates, use digital cameras to capture plate images and have automated incubators.

New analytical techniques

- Also, catalyzing progress in this area, allowing highly sensitive measurements that return rapid .They made based on the 3 mechanisms:

- **Single-Cell Methods for Bacterial Analysis**
- **Microfluidic Approaches for Rapid Bacterial Identification**
- **Ultrasensitive Detection Approaches**

Single-Cell Methods for Bacterial Analysis

- Introduced rapid monitoring changes in growth on a cell-by cell level.
- Single-cell **microbioreactors and microscale** incubators provide a means to culture bacteria in **very small volumes**.
- New types of single-cell analysis methods allow **rapid readout** of bacterial viability.

Cont'd

- **Advanced** single-cell systems are:
- No need to laboratory cultivation as very small numbers of bacteria can be analyzed.
- By using near-infrared wavelengths of light, cells could be easily handled.
- A high **throughput approach that tracks the morphology**(filamentous) of bacterial cells(and viruses) as they are exposed to different antibacterial drugs (**Fig.1**).
- Proposed as a valuable supplement to the efficient identification of novel microbial species and the accurate interpretation of the metagenomics and metatranscriptomics results.

Fig1

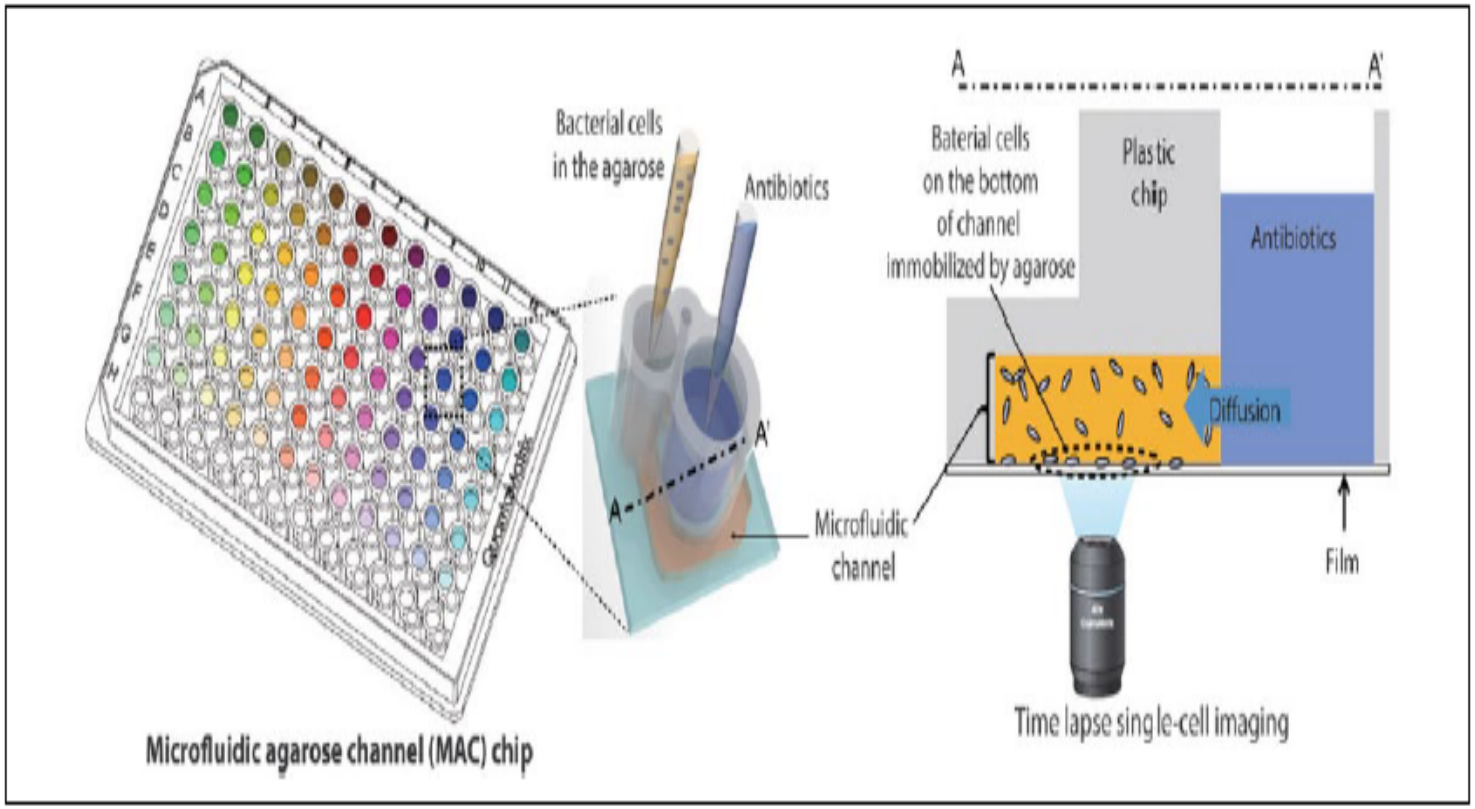

Figure 1. A high-throughput agarose channel chip for analysis of antibiotic susceptibility. Bacterial cells embedded in agarose are deposited within the chip, and an adjacent well contains an antibiotic to be tested. Bacterial morphology is monitored using time-lapse bright-field microscopy to gauge the response of the cells to different antimicrobials. Reprinted from Choi et al.[24] with permission.

Using this technique, 189 clinical isolates were analyzed, including cultures of *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus* spp. The set of bacteria tested included specimens exhibiting extended spectrum β-lactamase activity, imipenem resistance, methicillin resistance, and vancomycin resistance, and the isolates were exposed to a variety of antimicrobial agents. The morphological analysis was conducted in

Note: to comparison broth microdilution as gold standard, the discrepancy was less than 10%. It delivers results under 3 h as opposed to the 66 h required using conventional methods.

Microfluidic Approaches for Rapid Bacterial Identification

- Major advance are :

✓ The **miniaturization of biological assays.**

✓ The ability **to process and analyze biological and clinical samples is central to the automation** of bacterial identification.

✓ **Antibiotic resistance profiling**

- Turnaround times of just **a few hours**
- The ability to process **whole-blood samples**.

Cont'd

- **Efficiencies of bacterial isolation from blood cells is (>65%),** even when present at low abundance.
- To speed analysis, this approach was coupled with **quantitative RNA detection** using the commercially available Nanostring (Nanostring, Seattle, WA, USA) technology.
- **Workflow could be completed in 8 h (Fig2)**.

- **Note:** While this turnaround time is longer than for some of the other systems described above, **it represents a complete sample-to-answer protocol that enables the analysis of whole blood.**

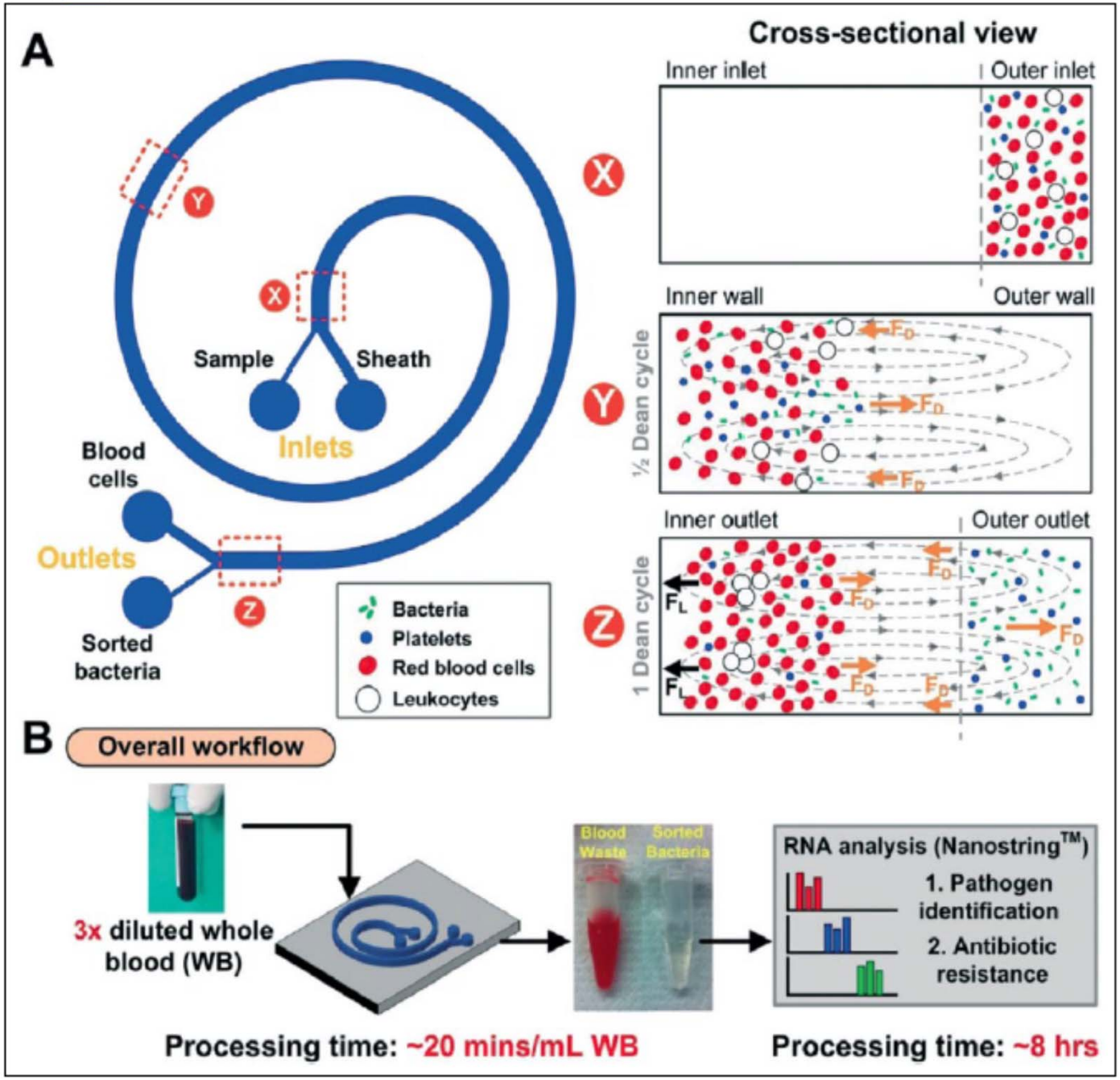

Figure 2. Inertial microfluidics for bacterial isolation and analysis. (**A**) A spiral microchannel device for inertial microfluidic separation of bacteria from blood. Larger hematologic cells experience stronger inertial lift forces that cause them to accumulate at the inner wall of the device, while smaller bacteria cells will proceed to the outer wall and outlet. (**B**) Overall workflow for the use of the device to isolate bacteria from whole blood. Reprinted with permission from Hou et al.[43]

Ultrasensitive Detection Approaches

- Conventional detection systems rely on optical density measurements, which are not very sensitive.
- A variety of electrophoretic, electrochemical, mechanical, and mass spectrometry– based systems have been developed for this application that may speed the diagnosis and identification of antibiotic resistant bacteria.

Cont'd

- It can be used to identify specific bacteria types, resistance enzymes, and successfully to analyze a variety of bacterial strains.
- **MALDI-TOF** can also be used to analyze bacterial isolates for the ability to hydrolyze antibiotics to detect antibiotic resistance.
- MALDI-TOF MS is widely used because of its high accuracy and speed of analysis.

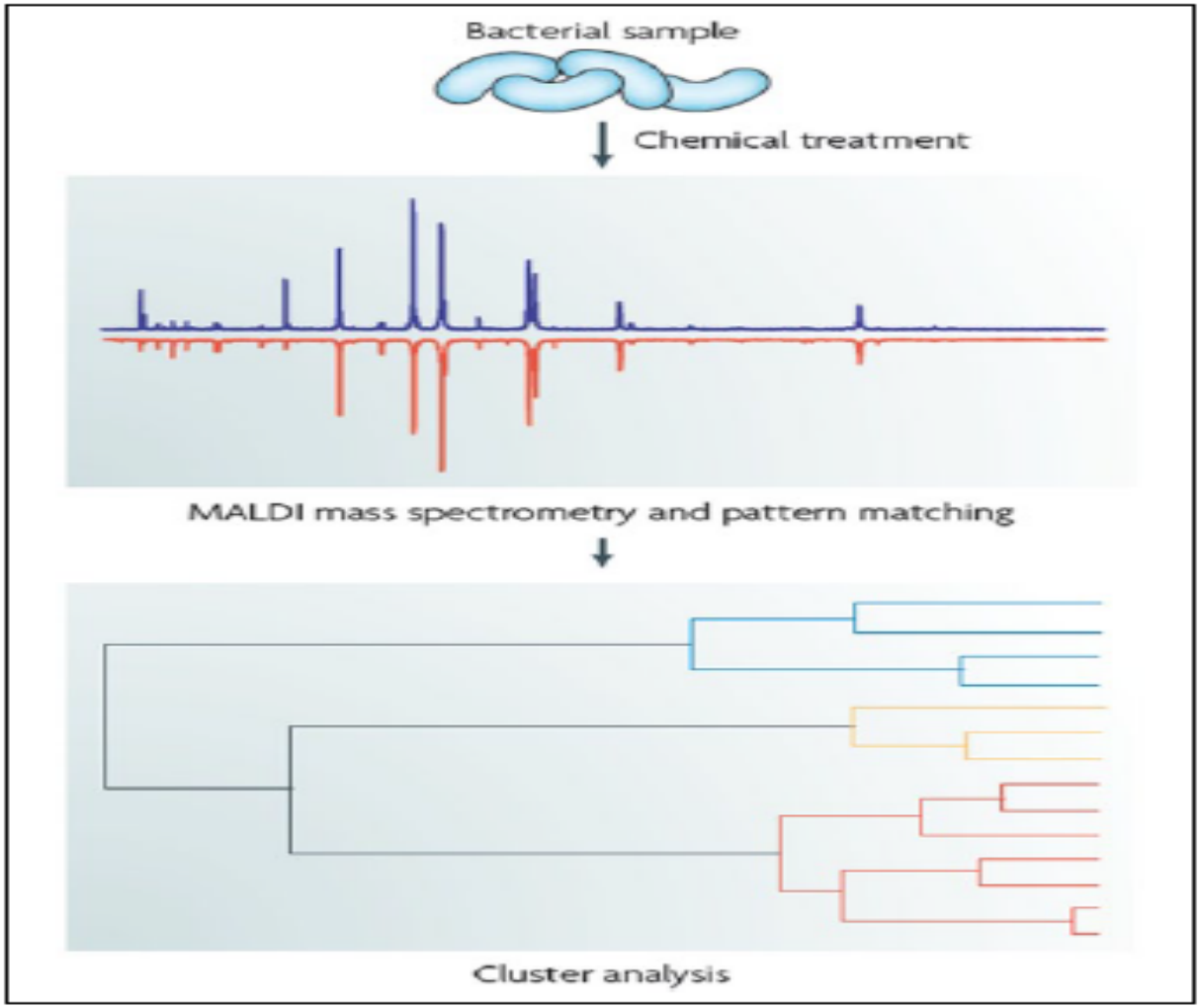

Figure 5. Matrix-assisted laser desorption (MALDI) mass spectrometry for bacterial identification. Mass spectrometry can be used to identify bacteria and their resistance patterns via the profiling of proteins or nucleic acids. Spectral fingerprinting combined with cluster analysis can enable highly specific bacterial characterization. Reprinted with permission from Sauer and Kliem.[81]

Summary

- A variety of new approaches are being deployed to combat antibiotic resistance by providing faster methods for bacterial identification and profiling.
- **Single-cell methods** have been developed that compare favorably to **gold-standard** microbiological tests,
- **New microfluidic** approaches are also producing promising advances that will speed test turnaround times.
- The development of **new analytical methods** that can detect bacteria faster using mechanical and electrochemical transducers will bring enhanced sensitivity to this problem.
- While all of these techniques face challenges related to streamlining sample preparation and system integration, it is anticipated that these translational barriers will be addressed as technologies mature.

Conclusion

- Despite of limitations which were mentioned to new methods, need to rapid techniques in clinical microbiology is mandatory. Excellent communication between research teams can lead the engineers and scientists to ban the risks and barriers.

References

- 1- Biochemistry 2018 Jan 16;57(2):209-215. doi: 10.1021/acs.biochem.7b00839. Epub 2017 Nov 10.
- 2- SLAS Technology 2017, 22(2): 113–121. DOI: 10.1177/2211068216680207
- 3- PNAS , December, 2018 ; 115 (51) : 12887–12895
- 4- Front Microbiol. 2017; 8: 1831. doi: 10.3389/fmicb.2017.01831
- 5- 4- Front Microbiol . 2016;7 , 1410 doi: 10.3389/fmicb.2016.00410

Thanks for your attention