Journal of Microbiological Methods 139 (2017) 48–53

Contents lists available at ScienceDirect

Journal of Microbiological Methods

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

Rapid separation of very low concentrations of bacteria from blood

Clara M. Buchanan[a], Ryan L. Wood[a], Taalin R. Hoj[b], Mahsa Alizadeh[a], Colin G. Bledsoe[a], Madison E. Wood[b], Daniel S. McClellan[a], Rae Blanco[a], Caroline L. Hickey[a], Tanner V. Ravsten[a], Ghaleb A. Husseini[c], Richard A. Robison[b], William G. Pitt[a,*]

[a] Chemical Engineering Department, Brigham Young University, Provo, UT, USA
[b] Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT, USA
[c] Chemical Engineering Department, American University of Sharjah, Sharjah, United Arab Emirates

ARTICLE INFO

Keywords:
Blood sepsis
Bacterial infection
Rapid diagnosis
E. coli
Carbapenem-resistant enterobacteriaceae
Sedimentation

ABSTRACT

A rapid and accurate diagnosis of the species and antibiotic resistance of bacteria in septic blood is vital to increase survival rates of patients with bloodstream infections, particularly those with carbapenem-resistant *enterobacteriaceae* (CRE) infections. The extremely low levels in blood (1 to 100 CFU/ml) make rapid diagnosis difficult. In this study, very low concentrations of bacteria (6 to 200 CFU/ml) were separated from 7 ml of whole blood using rapid sedimentation in a spinning hollow disk that separated plasma from red and white cells, leaving most of the bacteria suspended in the plasma. Following less than a minute of spinning, the disk was slowed, the plasma was recovered, and the bacteria were isolated by vacuum filtration. The filters were grown on nutrient plates to determine the number of bacteria recovered from the blood. Experiments were done without red blood cell (RBC) lysis and with RBC lysis in the recovered plasma. While there was scatter in the data from blood with low bacterial concentrations, the mean average recovery was 69%. The gender of the blood donor made no statistical difference in bacterial recovery. These results show that this rapid technique recovers a significant amount of bacteria from blood containing clinically relevant low levels of bacteria, producing the bacteria in minutes. These bacteria could subsequently be identified by molecular techniques to quickly identify the infectious organism and its resistance profile, thus greatly reducing the time needed to correctly diagnose and treat a blood infection.

1. Introduction

Persistent overprescription and misuse of antibiotics over past decades has contributed to the evolution of multiple strains of bacteria resistant to even last-line antibiotics such as carbapenems and colistin (Sprenger and Fukuda, 2016; Papp-Wallace et al., 2011). This in turn has led to an increase in septic infections of resistant bacteria in patients (Martin, 2012; Levine et al., 1999). A rapid and accurate diagnosis of sepsis is vital to increase survival rates of those with bloodstream infections, particularly for those with carbapenem-resistant *enterobacteriaceae* (CRE) infections (Patel et al., 2008). Sepsis is a threat in the United States and worldwide, with total annual costs of $16.7 billion nationally and up to 19 million yearly cases across the globe (Angus et al., 2001; Adhikari et al., 2010). Despite the extensive research that has gone into preventing and treating blood infections, CRE-related sepsis is still associated with mortality rates as high as 50% (Patel et al., 2008). The extremely low levels of bacteria (1 to 100 cells ml^{-1}) found in a septic patient's blood increase the difficult challenges of rapid diagnosis (Reimer et al., 1997). Currently, hospitals initially respond by treating the patient with broad-spectrum antibiotics, which is often expensive, sometimes inadequate, and unfortunately contributes to increasing the prevalence of antibiotic resistance (Anonymous, 2013). The standard clinical procedure involves a blood culture followed by an assay, which typically takes at least 12–24 h to perform (Diekema and Pfaller, 2013). Since survival rates associated with CRE sepsis drop by as much as 9% each hour that the infection remains untreated (Garnacho-Montero et al., 2006), it is paramount that more rapid methods are developed so that the infectious organism and its resistance profile can be correctly identified as quickly as possible.

Researchers have developed various novel methods to identify bacteria in septic blood, but many of these processes may be difficult to quickly and effectively implement because of cost, efficiency, time, or limits of detection. Chemical and magnetic capture techniques have shown promising results with high bacterial concentrations ($\sim 10^3$ to 10^4 CFU/ml) and high removal efficiencies (Shen et al., 2016; Herrmann et al., 2015; Kang et al., 2014a). Drawbacks of these capture

* Corresponding author at: 350 Clyde Bldg., Brigham Young University, Provo, UT 84602, USA.
E-mail address: pitt@byu.edu (W.G. Pitt).

http://dx.doi.org/10.1016/j.mimet.2017.05.004
Received 28 March 2017; Received in revised form 5 May 2017; Accepted 5 May 2017
Available online 08 May 2017
0167-7012/

methods include that the binding agents must be specific to the bacteria, there must be a large number of beads to capture the low numbers of bacteria found in septic blood, and the process is often time-consuming (Pitt et al., 2016). Few of these techniques have been demonstrated on blood with colony forming units (CFUs) in the range of 10–100/ml. In addition, the materials required in magnetic and chemical separation of bacteria from blood are often associated with a high cost (Pitt et al., 2016). Microfluidic devices have also received attention in the literature for successfully separating red blood cells (RBCs) from bacteria or other smaller particles using red cell migration and particle focusing (Hou et al., 2016; Wu et al., 2009). These techniques typically employ small volumes of diluted blood, but could be scaled to higher blood processing rates using parallel flow devices (Pitt et al., 2016).

A high throughput of blood is necessary because the low concentrations of bacteria in a patient's blood require several milliliters of blood to be processed in order to collect enough bacteria for analysis. Additionally, the diagnostic process must produce rapid results because mortality increases rapidly with time. Finally, the technique must be reliable on the low numbers of bacteria found in a patient in order to have the detection sensitivity to be feasible in a clinical setting. Many of the published investigations involve experiments with thousands or millions of CFUs/ml – a high concentration that would rarely be found in a living person (Kreger et al., 1980; Leggieri et al., 2010; Yagupsky and Nolte, 1990). On the other hand, there are only a few investigations of bacterial separation from blood at clinically relevant concentrations of bacteria. For example, Kang et al. reported 77% recovery with 1 CFU/ml in blood using an Integrated Comprehensive Droplet Detection platform technology (Kang et al., 2014b). However, this was done on diluted blood. There are few methods shown to rapidly achieve this level of recovery on whole blood.

Our research group has developed a technique that successfully and inexpensively achieves rapid separation of bacteria from milliliter quantities of blood. However, those initial separation experiments ($\sim 10^6$ CFU/ml) were not performed at clinically relevant (low) concentrations of bacteria in a patient's blood (Alizadeh et al., 2017). Therefore in this present report, very low concentrations of bacteria (originally 6 to 200 CFU/ml) were separated from blood using sedimentation in a spinning hollow disk. Due to the low number of bacteria, the plasma (containing the bacteria and some residual RBCs) was vacuum-filtered instead of using dilution and plating as our research group had done previously. Following vacuum filtration, the CFUs on the filters were counted to calculate the percent of bacteria collected from the original sample of human blood spiked with *E. coli*. This technique effectively separated bacteria from blood with concentrations of bacteria in blood as low as 6 CFU/ml in one minute of spinning.

2. Materials and methods

2.1. Solutions

Phosphate buffered saline (PBS, without calcium) was made from salts purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Fair Lawn, NJ). PBS was sterilized by autoclaving.

Sodium dodecyl sulfate (SDS, Acros Organics in Fair Lawn, NJ) was dissolved into distilled deionized water (DDH2O) at ~50 °C and cooled to make a 15% stock solution.

Ethanol for disinfecting the spinning hollow disk was made by diluting 95% denatured ethyl alcohol (Fisher Chemical, Fair Lawn, NJ) with sterile water to a final concentration of 70% (vol/vol).

2.2. Preparing bacteria

Escherichia coli (*E. coli*, strain BL-21 Star DE3) was streaked from frozen culture onto a nutrient agar plate (DIFCO Sparks, MD) and incubated at 37 °C for 24 h. From this plate, a single colony was inoculated into 1 ml of nutrient broth (DIFCO Sparks, MD) in a sterile test tube. After shaking for 8 h at 200 rpm at 37 °C, this 1 ml bacteria suspension was transferred to 50 ml nutrient broth in a 250-ml sterile shaker flask. This flask was shaken for another 16 h at 200 rpm and 37 °C. The bacteria were washed twice by centrifugation (Horizon Model 642E, Fisher HealthCare, Fair Lawn, NJ, 1575 × *g* for 10 min at room temperature) and resuspension in sterile PBS. The bacterial concentration was estimated by optical density at 600 nm using a spectrophotometer. The bacteria were diluted with sterile PBS to form a stock solution of the desired concentration.

2.3. Preparing blood for spinning

Following an IRB-approved protocol, and after informed consent of volunteers, blood was collected on the day of the experiment from healthy human donors into 10-ml EDTA anti-coagulant tubes (Vacutainer #366643, Becton Dickinson, Franklin Lakes, NJ). Immediately before experiments, the tubes were inverted several times by hand and 8.0 ml of blood was pipetted into another sterile test tube. A volume of 100 µl of the *E. coli* stock solution was added to the blood and mixed by lightly vortexing for 10 s.

2.4. Separation by sedimentation

To separate the plasma and bacteria from the RBCs and white blood cells (WBCs), a 12-cm-diameter spinning hollow disk was used (see Fig. 1). These acrylic disks were designed and printed in our lab using a rapid prototyping instrument (Object 30 Prime, Stratasys, Eden Prairie,

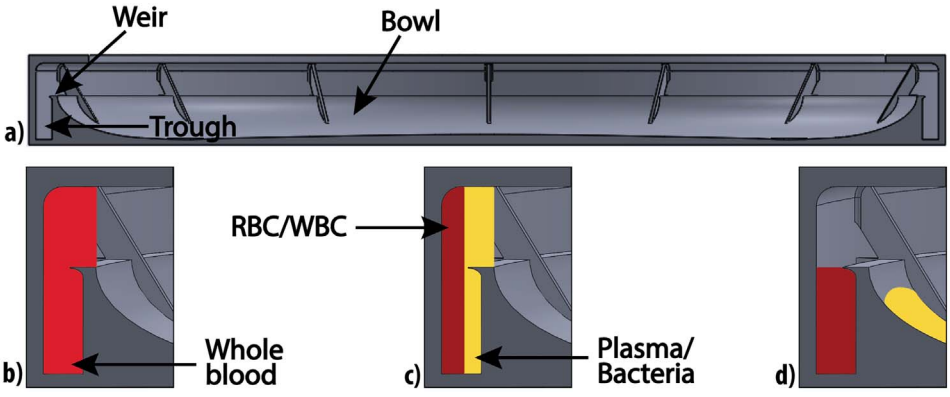

Fig. 1. Schematic of spinning blood in a hollow disk. a) Cross section of empty disk showing bowl, trough and weir. b) Upon spinning, blood is spun to the periphery of the disk. c) After 45 s at 3000 rpm, the RBCs and WBCs have sedimented quickly to form an outer layer, while most of the bacteria remains in the plasma layer. d) At conclusion of slow deceleration, the cells slide down into the trough while the plasma containing bacteria flows over the edge of the weir into the bowl where it is collected.