Ideal Growth Conditions for *Elizabethkingia meningoseptica*

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Abstract

Elizabethkingia meningoseptica is a Gram-negative pathogen that demonstrates extreme multi-drug resistance. To date, there have been no studies examining which bacteriological media is best to grow this organism in. Out of 5 different media we found that *E. meningoseptica* grew best in heart infusion broth and both 30°C and 37°C. Due to the presence of multiple genes encoding beta -lactamases and carbapenemases in the *E. meningoseptica* genome, we also wanted to test if this organism could grow in the presence of the carbapenem, imipenem. As expected, the organism grew relatively well at a low imipenem concentration (8 μg/mL), compared to higher concentrations of this drug. This latter experimental data will be used to define imipenem concentration to be used to characterize the imipenem-induced transcriptome of this organism in order to identify which of the ~ 20 beta-lactamase genes are induced by imipenem challenge.

Keywords: *Elizabethkingia meningoseptica*, imipenem

Introduction

Elizabethkingia meningoseptica is a Gram-negative pathogen that was first isolated from a neonatal meningitis patient in 1958 (Brody. *et al.* 1958). *E. meningoseptica* is ubiquitous, and is found in plants, soil and other water sources (Gokce *et al.* 2012) as well as in chlorhexidine solutions (Coyle-Gilchrist *et al.* 1976) and aerosol solutions containing antibiotics (Oie and Kamiya 1995). *E. meningoseptica* demonstrates a multi-drug resistant phenotype and contains characterized metallo-beta-lactamase and extended spectrum beta-lactamase genes that confer beta-lactam resistance (Cartwright *et al.* 2010, Yum *et al.* 2010).

Elizabethkingia has been speciated into a number of species, including *E. miricola* and *E. anopheles*, which have been isolated from the Russian Mir space station (Li *et al.* 2003) and the mid-gut of a mosquito (Kampfer *et al*. 2011), respectively. Due to *Elizabethkingia's* ability to grow in many diverse environments, it is important to determine the optimal growth conditions in order to culture the bacterium in the laboratory. In addition, *E. meningoseptica* demonstrates resistance to imipenem, a beta-lactam antibiotic used to treat serious bacterial infections (Kesado *et al*. 1980). In 2012, *E. meningoseptica* was isolated from patients in a London adult critical care unit that demonstrated resistance to this drug (Moore *et al.* 2016). More recently, in Wisconsin during December of 2015, an outbreak of *E. anopheles* led to 56 confirmed infections and 18 deaths. Therefore, continuing research to further characterize *E. meningoseptica* growth parameters is imperative to determine optimum isolation procedures.

The aim of this study is to determine the optimal growth conditions for *E. meningoseptica* within the laboratory. Additionally, we want to determine a concentration of imipenem that we can use to investigate the expression of the beta-lactamase genes in *E. meningoseptica*.

Methods and Materials

Materials

The growth media used was as follows: Mueller Hinton broth (Becton Dickinson, Franklin Lakes, New Jersey), heart infusion broth (Becton Dickinson, Franklin Lakes, New Jersey), nutrient broth (Becton Dickinson, Franklin Lakes, New Jersey), Luria broth (Becton Dickinson, Franklin Lakes, New Jersey), and tryptic soy broth (EMD Millipore, Darmstadt, Germany). Imipenem powder was purchased from Gold Bio, St. Louis, Missouri.

Growth analysis

Overnight cultures (3 mL) with the various media were initiated with a single colony of *E. meningoseptica* and grown at either 30° C or 37° C (200 rpm) for ~ 12 hrs. The overnight cultures were then used to inoculate a 20 mL aliquot of media in a 50 mL Erlenmeyer flasks to reach a starting OD600 of 0.01. Growth was then monitored by measuring the OD600nm every hour for 8 hours.

Growth analysis with imipenem

These experiments were conducted in heart infusion media as above with the exception of adding imipenem to reach final concentrations of 8 μg/mL, 16 μg/mL and 32 μg/mL to the growth medias. Growth flasks were incubated at 30° C or 37° C at 200 rpm and growth was monitored every hour for 8 hours.

Results

The study showed that *E. meningoseptica* grew optimally in heart infusion broth and least optimally in

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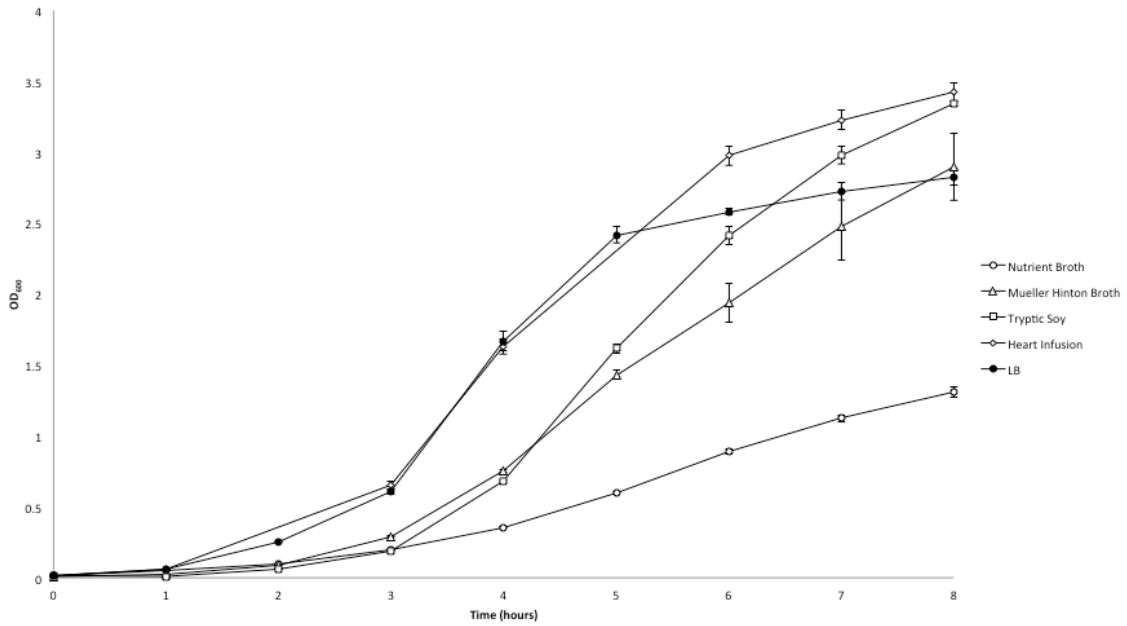

Figure 1. *Average E. meningoseptica growth at 37°C (n = 3; bars represent standard error).*

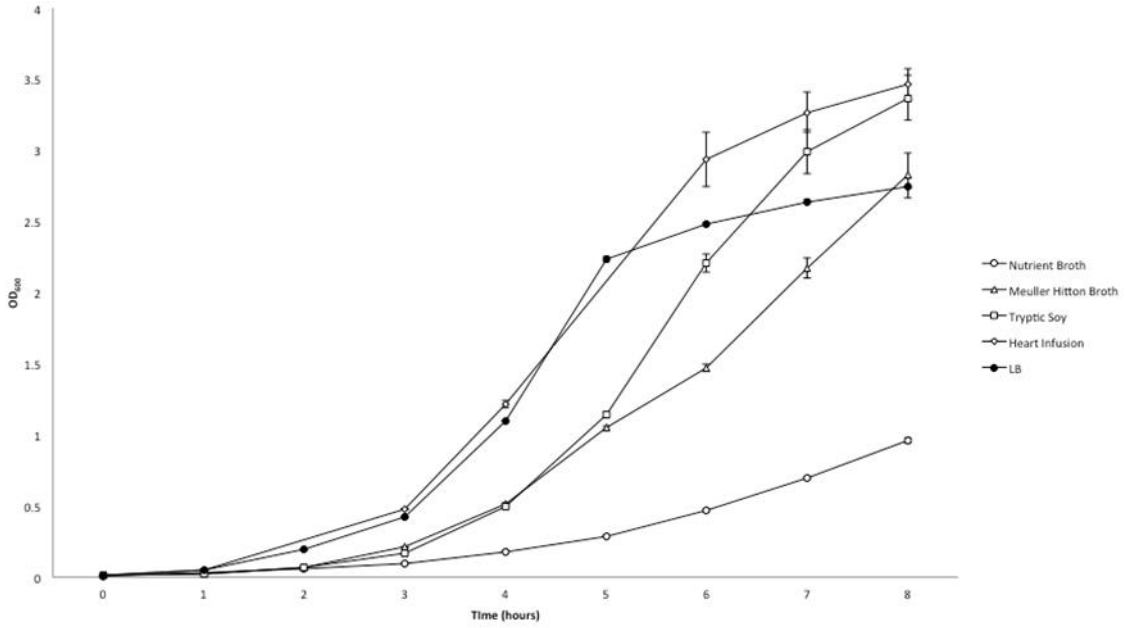

Figure 2. *Average E. meningoseptica growth at 30°C (n = 3; bars represent standard error).*

nutrient broth (Figure 1 and Figure 2) at both 30° C and 37° C. Additionally, we found that *E. meningoseptica* grew best at 37° C in all growth media tested (Figure 1 and Figure 2). The organism grew relatively well in 8 µg/mL imipenem at both temperatures over an 8 hour time period (Figure 3 and Figure 4), and minimal growth was observed after 24 hour growth in 16 and 32 µg/mL imipenem (data not shown).

Conclusions

Our data demonstrates that *E. meningoseptica* can grow in many different media at two temperatures, and that heart infusion media best supports growth of this organism. *E. meningoseptica* can grow in the presence of 8 µg/mL and higher concentrations of imipenem, which is attributed to the presence of the beta-lactamase genes in the organism's genome. We hypothesis that the lag phase in the growth of *E. meningoseptica* in the presence of 8 µg/mL imipenem is indicative of beta-lactamase gene induction.

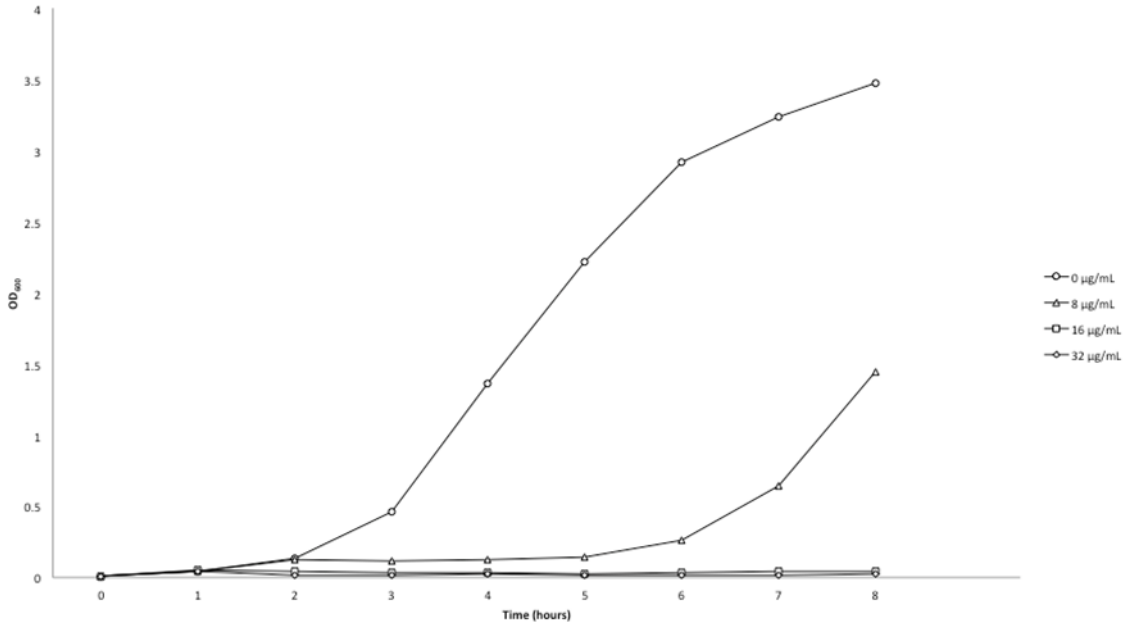

Figure 3. *E. meningoseptica growth at 37∘C with varying concentrations of imipenem.*

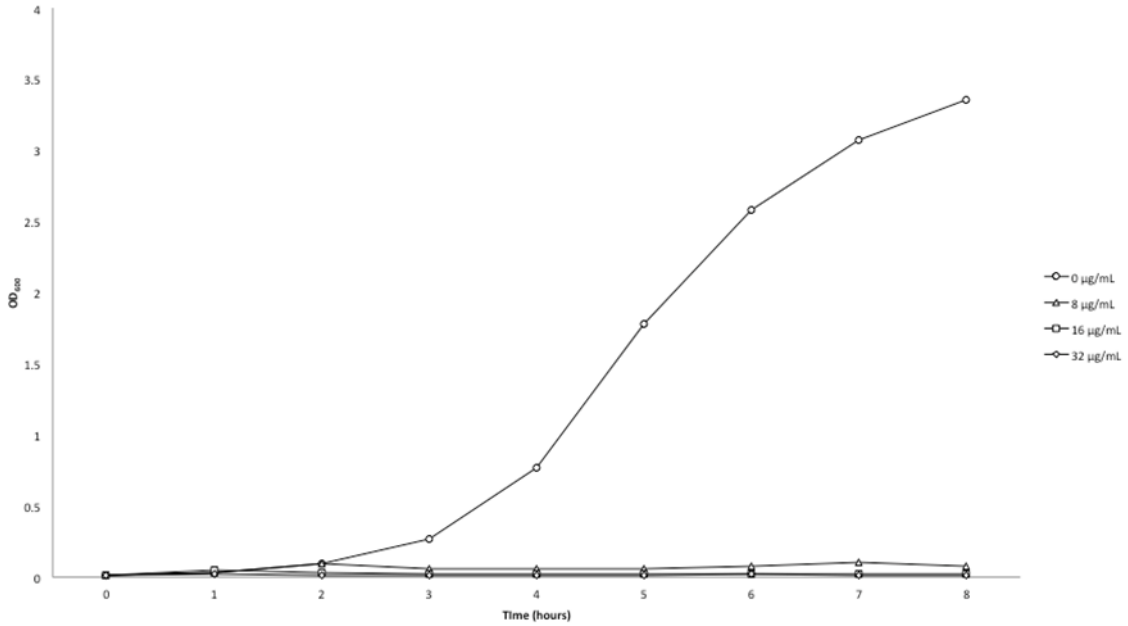

Figure 4. *E. meningoseptica growth at 30∘C with varying concentrations of imipenem.*

More work is required to determine an optimal concentration of imipenem to investigate the imipenem-induced transcriptome of *E. meningospetica*.

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