Effect of vancomycin and ceftazidime on Biofilm formation in *Elizabethkingia*

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Introduction

Elizabethkingia infection primarily occurs in neonates and other immunocompromised individuals. Infections with these organisms are associated with a high mortality rate in part, due to their expression of multiple antibiotic resistance[1,2]. Vancomycin has been used to treat *Elizabethkingia* infections, but the treatment outcomes compared to *in vitro* vancomycin susceptibility testing have produced conflicting results[3]. *Elizabethkingia* can also form biofilms, which are groups of bacterial cells that assemble into a matrix, creating a defensive barrier against antibiotics and the immune response[4,5]. Biofilm formation may be altered when cells are exposed to concentrations of antibiotics that are too low to kill the organism, but this has not been tested in *Elizabethkingia*. Our study seeks to test the effects of vancomycin and ceftazidime on biofilm formation of all known *Elizabethkingia* species.

Methods

Growth Conditions:
Heart infusion agar (HIA) supplemented with 5% defibrinated rabbit blood was used to grow and preserve working stocks of the bacteria. A single colony from the working stocks was placed in 3 ml Mueller-Hinton broth (MHB) and incubated at 37°C (24 h, 200 rpm) to make an overnight culture.

Minimum inhibitory and bactericidal (MIC/MBC) assays:
MICs and MBCs for each type strain were determined using the microtiter method following standard CLSI guidelines[6].

Biofilm Growth Conditions:
Parent strains for each species (Table 1) were tested in triplicate on 96 well microtiter plates with positive controls and the appropriate concentration of each antibiotic (Tables 2 and 3). Overnight cultures were diluted to an optical density at 600 nm (OD_{600nm}) of 0.01 and100 µl of this diluted culture was used to inoculate each well. Plates were incubated at 37°C for 24, 48, or 72 hr.

Crystal Violet Biofilm Assay:
Following incubation the OD_{600nm} was recorded, growth media was removed, and each well was rinsed with 100 µl autoclaved diH_2O to remove any non-adherent cells. After washing, the biofilms were fixed with 100 µl 100% methanol for 15 min, and allowed to air dry until all methanol had evaporated. To visualize biofilms,100 µl of 0.2% crystal violet solution was added to each well for 5 min. Crystal violet was then removed and plates were thoroughly rinsed with diH_2O (Figure 1). Biofilm formation was assessed by adding100 µl 95% ethanol to each well and then read at OD_{570nm} with a BioTek Synergy H1 plate reader. Wells were considered positive for biofilm formation if the OD_{570} was ≥ mean OD_{570nm} + 3 standard deviations of 3 uninoculated control wells.

Results

Table 1. MICs and MBCs (µg/ml) of the 6 type strains of *Elizabethkingia*.

Strain	Species	Vancomycin		Ceftazidime	
		MIC	MBC	MIC	MBC
R26	*anophelis*	8	16	>256	>256
ATCC 33958	*bruuniana*	4	8	16	>256
KC1913	*meningoseptica*	64	64	>256	>256
G4071	*miricola*	4	8	>256	>256
G4070	*occulta*	8	16	256	>256
G4122	*ursingii*	2	4	4	16

Table 2. Biofilm formation for each type strain in the presence of vancomycin. Each strain was challenged at ½ MIC.

Isolate	Species	Vancomycin Concentration (µg/ml)	24 hr		48 hr		72 hr	
			Control	Treatment	Control	Treatment	Control	Treatment
R26	*anophelis*	4	3	3	3	3	3	3
ATCC 33958	*bruuniana*	2	2	3	3	3	3	3
KC1913	*meningoseptica*	32	3	0	3	1	3	3
G4071	*miricola*	2	1	3	3	3	3	3
G4070	*occulta*	4	3	3	3	3	3	3
G4122	*ursingii*	1	3	3	1	1	3	3

Table 3. Biofilm formation for each type strain in the presence of ceftazidime. Each strain was challenged at a concentration of 32 µg/ml.

Isolate	Species	24 hr		48 hr		72 hr	
		Control	Treatment	Control	Treatment	Control	Treatment
R26	*anophelis*	3	3	3	3	3	3
ATCC 33958	*bruuniana*	3	3	3	3	3	3
KC1913	*meningoseptica*	2	2	3	3	3	3
G4071	*miricola*	3	2	2	3	3	3
G4070	*occulta*	3	3	3	3	3	3
G4122	*ursingii*	3	0	3	1	3	0

Methods Continued

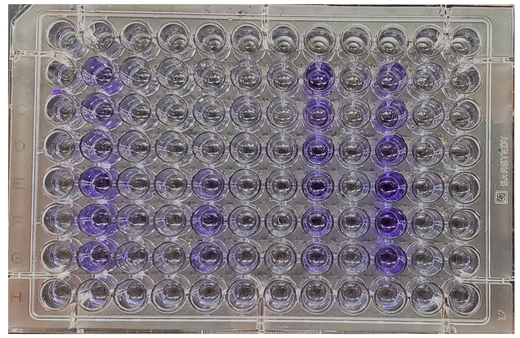

Figure 1. 96 microtiter plate after rinsing out excess crystal violet.

Results

- **The isolates demonstrated varied MICs and MBCs for vancomycin and ceftazidime (Table 1).**
- **All species were able to form biofilms in drug free media (Tables 2 and 3). Some controls for strains G4071 and G4122 however did not form biofilms, which we suspect is due to experimental error (Tables 2 and 3).**
- **With the exception of KC1913 (with vancomycin at 24 and 48 hr) and G4122 (with ceftazidime at all time points examined), neither vancomycin (@ 0.5 X the MIC concentration) nor ceftazidime impacted biofilm formation at any time point examined. (Tables 2 and 3).**
- **KC1913 did however form biofilms after 72 hr, suggesting the selection for vancomycin resistance (Table 2).**
- **The concentration of 32 ug/ml ceftazidime used on G4122 (Table 3) was higher than the observed MIC, and it is likely that the lack of biofilm formation is due cell death.**

Conclusions

- **Our results demonstrates that vancomycin can impact biofilm formation in *Elizabethkingia meningoseptica and ceftazidime (> MIC) can impact biofilm formation in Elizabethkingia ursingii*.**
- **Overall though, vancomycin seems ineffective in reducing biofilm formation in the *Elizabethkingia*.**

References

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