Resistance to the Minocycline-Rifampin-Chlorhexidine (MRCH) combination does not emerge in biofilms of Tetracycline and Rifampin resistant bacteria grown on MRCH catheters depleted below antimicrobially effective concentrations.

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Abstract

Background: Central Line Associated Bloodstream Infections (CLABSIs) remain a significant medical problem for critically ill cancer patients who required catheters for extended durations. Minocycline (M) -Rifampin (R) loaded catheters have shown the greatest impact on reducing CLABSIs; however, there is a risk for developing antibiotic resistant organisms when exposed to catheters whose concentration becomes depleted below antimicrobially effective levels due to extended indwells. Chlorhexidine (CH) and M-R combination catheters (MRCH) have been proposed as a next generation catheter with improved performance. Here we studied whether bacteria that were Tetracycline and Rifampin resistant became resistant to MRCH when allowed to form biofilms on MRCH catheters depleted below antimicrobially effective MRCH concentrations.

Methods: Minimum inhibitory concentrations (MICs) of Tetracycline and/or Rifampin resistant stock isolates were measured by standard microbroth dilution methods. MRCH catheters were depleted to below antimicrobially effective concentrations by soaking in serum for 6 weeks. The resistant bacteria were then allowed to form biofilm for 24 hrs on the depleted catheters in broth. Following 24 hour incubation the adherent (breakthrough) bacteria were removed by sonication and MICs were remeasured. The same organisms grown on non-antimicrobial catheters were used as controls.

Results: MICs (ug/mL) of the organisms against each agent and the combination are tabulated below:

Conclusion: The M and R resistant bacteria did not develop in vitro resistance to the MRCH combination after forming biofilms on MRCH catheters depleted below antimicrobially effective concentrations.

Bacteria	History	Rifampin	Minocycline	CH	MRCH
Klebsiella penumoniae (AR542)	Stock organism	16	2	2	2
	MRCH biofilm	16	2	2	2
Enterobacter cloacae (AR544)	Stock organism	8	4	1	2
	MRCH biofilm	8	4	2	2
Staphylococcus aureus (AR 219)	Stock organism	>32	16	1	1
	MRCH biofilm	>32	16	1	1
Staphylococcus aureus (VISA) (AR 722)	Stock organism	4	0.5	1	0.5
	MRCH biofilm	4	0.5	1	0.5
Enterococcus faecium (VRE) (AR 579)	Stock organism	8	16	2	2
	MRCH biofilm	8	16	2	2

Introduction

When microorganisms are exposed to antibacterial agents or coated medical devices that prevent microbial growth, the potential of developing antimicrobial resistance increases. To assess the potential for induction of mutational antimicrobial resistance, a previous study repeatedly exposed many pathogenic organisms to sub-inhibitory concentrations of Minocycline, Rifampin, and Chlorhexidine through multiple passages where changes in MICs were measured following each passage. There was no evidence that organisms developed mutational resistance to the triple combination over 20 passages. Here, we assessed the potential for development of resistance by horizontal gene transfer in pathogenic biofilms on catheter surfaces when multidrug resistant organisms were exposed to subinhibitory concentrations of MRCH on the catheter surfaces following depletion of M, R and CH content in the catheters by elution in serum over prolonged simulated catheter indwells.

Methods

Biofilm Colonization Model- Minocycline/Rifampin + Chlorhexidine (M/R+CH) is a combination of two antibiotics and an antiseptic that was developed as a new antimicrobial treatment to inhibit biofilm colonization of various Gram-negative and Gram-positive organisms. M/R+CH catheters were cut into 1 cm segments, placed in 1mL of human donor plasma for 24 hours, removed, and eluted in newborn calf serum for 6 weeks (4 weeks for gram-negative bacteria). Gram-negative and gram-positive bacteria were inoculated and allowed to attach, colonize and form biofilm for 24 hours at 37C. The catheter segments were placed in 1mL of 0.9% of saline and washed with shaking for 30 minutes to remove non-adherent bacteria. After washing, segments were sonicated in 1mL of neutralizer for 15 minutes. Sonicate were serially diluted and plated onto trypticase soy agar + 5% sheep blood. Plates were incubated for 24 hours at 37C and counted for viable colonies.

Organism tested-

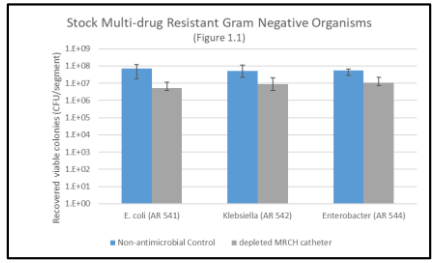
- Staphylococcus aureus (AR 219)
- Staphylococcus aureus (AR 722)
- Enterococcus (AR 579)
- Escherichia coli (AR 541)
- Klebsiella pneumonie (AR 542)
- Enterobacter cloacae (AR 544)

Minimum Inhibitory Concentration (MIC) Model- The organisms recovered from Tecoflex control and breakthrough M/R+CH on the trypticase soy agar + 5% sheep blood from the Biofilm Colonization test and the baseline organisms were used to assess in vitro activity of M/R+CH against Gram-negative and Gram-positive isolates by standard CLSI microbroth dilution methods. Rifampin, Minocycline, and Chlorhexidine individually were used as comparators.

Conclusion

The viable microbial concentrations in biofilms formed on depleted MRCH catheters (Figures 1.1 and 1.2) were 1-2 logs below the concentrations in biofilms formed on non-antimicrobial catheters. This indicates that the catheters depleted of antimicrobial agents by extended soaking for several weeks in serum (to simulate long indwells in patients) retained slight antimicrobial activity but insufficient activity to prevent attachment, colonization and robust biofilm formation on the catheter surfaces. The MIC measurements shown in Tables 1.1 and 1.2 for each stock organism and its breakthrough comparators are within normal experimental variability indicating the organisms in the biofilms did not develop further antimicrobial resistance to the MRCH combination from exposure to low concentrations of antimicrobial agents on the depleted catheter surfaces for an extended time. The absence of bacterial growth on non-depleted (fresh) MRCH catheters when challenged by the breakthrough organisms (Figures 2.1 and 2.2) demonstrates that fresh MRCH catheters retained efficacy against the same organisms that grew on depleted MRCH catheters. This verified that resistance to the MRCH combination was not acquired or transferred following biofilm formation on catheters presenting low (depleted) concentrations of MRCH.

Results



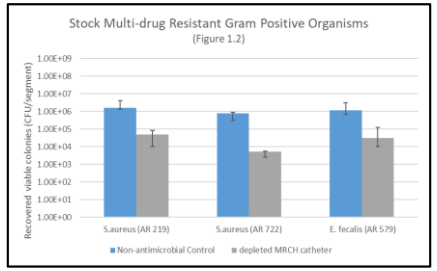


Figure 1.1- Biofilm colonization by resistant gram negative organisms on non-antimicrobial and depleted MRCH CVCs – MRCH catheters were depleted soaking in serum for 4 weeks and then exposed to resistant E. coli, Klebiella, and Enterobacter bacteria. Breakthrough biofilm growth on the 4-week depleted catheters was then quantitatively cultured to assess breakthrough biofilm microbial concentrations. Data is presented as median and range bars of 6 replicates.

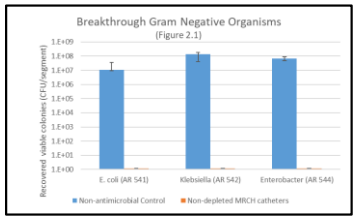
Figure 1.2- Biofilm colonization by resistant gram positive organisms on non-antimicrobial and depleted MRCH CVCs – MRCH catheters were depleted of their antimicrobial agents by soaking in serum for 6 weeks and then exposed to resistant Staphylococcus and Enterococcus bacteria. Breakthrough biofilm growth on the 6-week depleted catheters was then quantitatively cultured to assess breakthrough biofilm microbial concentrations. Data is presented as median and range bars of 6 replicates.

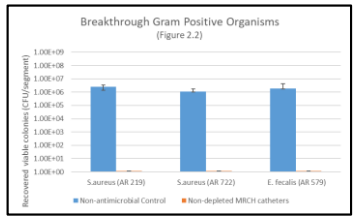
Organism	Type	Rifampin	Minocycline	CHX	MRCH
Escherchia coli (AR541)	Stock organism	2	1	0.5	1
	MRCH breakthrough	2	2	0.5	1
Klebsiella penumoniae (AR542)	Stock organism	16	2	2	2
	MRCH breakthrough	16	2	2	2
Enterobacter clocae (AR544)	Stock organism	8	4	1	2
	MRCH breakthrough	8	4	2	2

Table 1.1- MICs of gram negative organisms against Minocycline, Rifampin, Chlorhexidine and combinations - MICs were measured by microbroth dilution for the stock organism and the breakthrough organisms cultured from the depleted MRCH catheters. Differences beyond normal experimental variability were not seen between MICs of breakthrough organisms vs stock organisms indicating, development of resistance did not occur. MICs are presented as ug/mL.

Organism	Type	Rifampin	Minocycline	CHX	MRCH
Staphyloccocus aureus (AR219)	Stock organism	>32	16	1	1
	MRCH breakthrough	>32	16	1	1
Staphyloccocus aureus (AR722)	Stock organism	4	0.5	1	0.5
	MRCH breakthrough	4	0.5	1	0.5
Enterococcus (AR579)	Stock organism	8	16	2	2
	MRCH breakthrough	8	16	2	2

Table 1.2- MICs of gram positive organisms against Minocycline, Rifampin, Chlorhexidine and combinations - MICs were measured by microbroth dilution for the stock organism and the breakthrough organisms cultured from the 6-week depleted MRCH catheters. No difference was seen between MICs of breakthrough organisms vs stock organisms indicating development of resistance did not occur. MICs are presented as ug/mL.





Figures 2.1 and 2.2 - Biofilm colonization of breakthrough gram negative and gram positive organisms on non-depleted MRCH catheters – To verify that breakthrough organisms did not acquire resistance, Breakthrough organisms cultured from the depleted MRCH catheters were reused to challenge fresh (non-depleted) MRCH catheters. Colonization and biofilm formation was completely prevented on the non-depleted MRCH catheters verifying that there was no development of resistance following biofilm formation on catheters with very low antimicrobial concentrations in the depleted catheters. Data is presented as median and range of 3 replicates.