

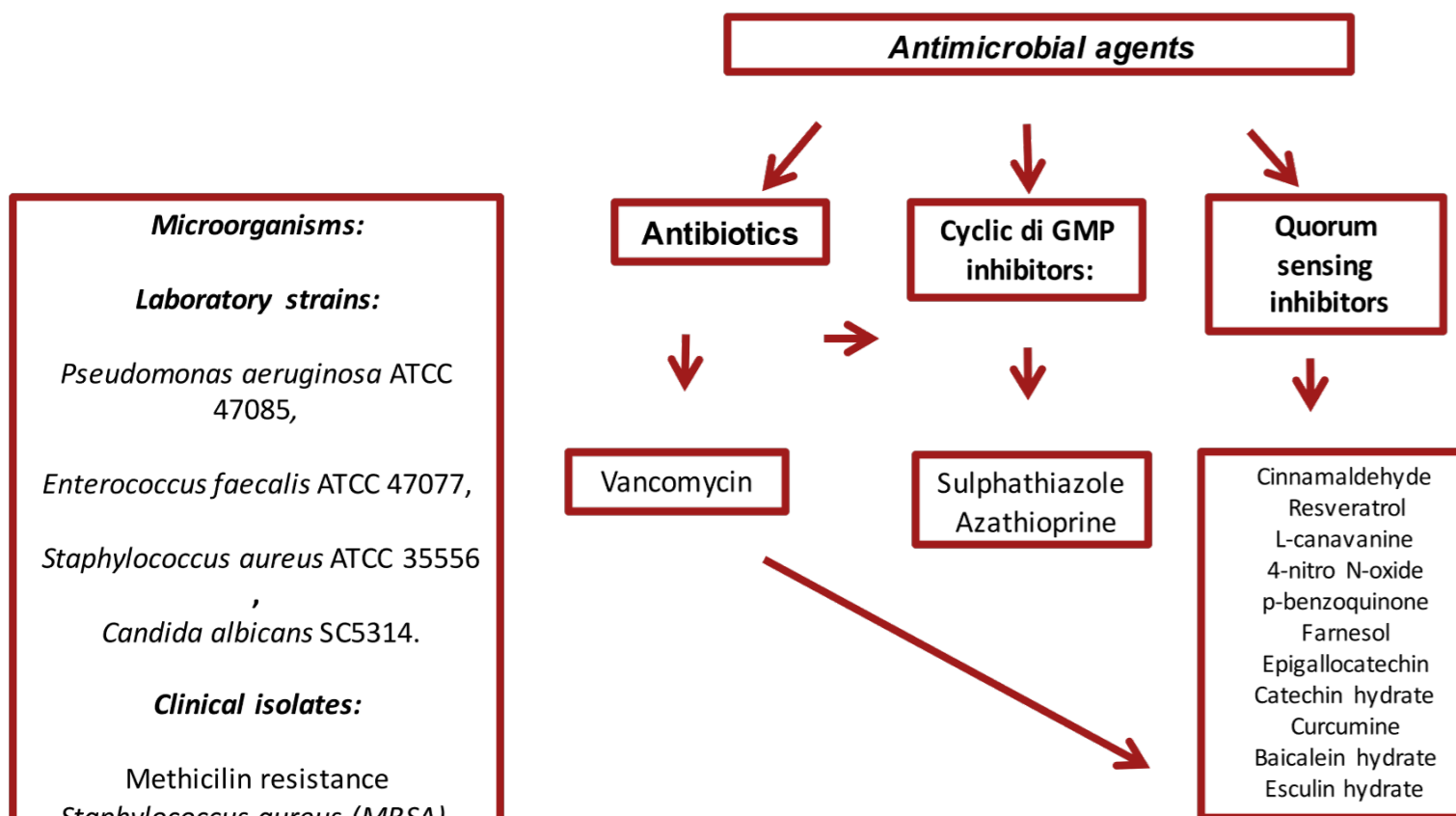


Didem Kart¹, Meral Sağıroğlu¹,
1 Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology

INTRODUCTION

Polymicrobial biofilms are common problem in nosocomial infections. *Staphylococcus aureus*, one of the major pathogen related with the biofilm infections, is an important trouble in hospitals. Quorum sensing is an alternative strategy in fighting biofilm infections and some natural quorum sensing inhibitors (QSI) were found successful to interfere with the infections. In our study polymicrobial biofilms were reproducibly grown, consisting of *S.aureus*, *P.aeruginosa*, *E.faecalis* and *C.albicans* in a 96 well microtiter plate. The antibiofilm effect of vancomycin in alone and combination with different QSIs derived from natural plants and two c-di-GMP inhibitors respectively were evaluated on mono and polymicrobial biofilm models of *S.aureus*.

MATERIAL-METHODS



- Microorganisms:**
- Laboratory strains:**
- Pseudomonas aeruginosa* ATCC 47085,
 - Enterococcus faecalis* ATCC 47077,
 - Staphylococcus aureus* ATCC 35556
 - Candida albicans* SC5314.
- Clinical isolates:**
- Methicilin resistance *Staphylococcus aureus* (MRSA),
 - Vancomycin resistance *Enterococcus faecium* (VRE)
 - Carbapenem resistance *Pseudomonas aeruginosa*

- ✓ Monomicrobial biofilm models of *P.aeruginosa* ATCC 47085 and a carbapenem resistance *P.aeruginosa* isolate and polymicrobial biofilm models consisting of *C.albicans*, *E.faecium*, *P.aeruginosa* and *S.aureus* were grown *in vitro*.
- ✓ Antibiofilm effect of the agents (indicated above) in alone and combinations were tested on the biofilm models by MBEC assay previously known as Calgary biofilm devices. MBIC (minimum biofilm inhibition concentration) and MBEC (minimum biofilm eradication concentration) value of the agents were determined with this assay.
- ✓ MIC values of antimicrobial agents were determined by broth dilution method and sub-mic values were used for biofilm analysis
- ✓ After treating with the antimicrobial agents , logarithmic reduction of *S.a* sessile cells in mono and polymicrobial biofilms were determined
- ✓ Anti-quorum sensing activity of the agents were determined by using *Chromobacterium violaceum* CV12472

RESULTS

Table 1: MIC, MBIC and MBEC results of *S.aureus* ATCC 35556 and MRSA isolate

	<i>S.aureus</i> ATCC 35556			MRSA		
	MIC	MBIC	MBEC	MIC	MBIC	MBEC
Vancomycin	-	≤0.5	4	-	4	4
V1	-	≤0.5	1	-	≥128	0.125
V2	-	≤0.5	8	-	≤0.0625	4
V3	-	≤0.5	2	-	4	4
V4	-	≤0.5	≥512	-	4	4
V5	-	1	1	-	4	4
V6	-	≤0.5	≥512	-	4	4
V7	-	≤0.5	1	-	≤0.0625	32
V8	-	≤0.5	1	-	4	16
V9	-	≤0.5	≥512	-	4	4
V10	-	≤0.5	≤0.5	-	8	16
V11	-	≤0.5	1	-	4	4
V12	-	≤0.5	≤0.5	-	≤0.0625	0.125
V13	-	≤0.5	≤0.5	-	128	≤0.0625
Curcumine	1024	8	8	1024	≥1024	16
Azathioprine	1024	32	16	1024	512	≥1024
Resvatrol	256	32	64	256	1024	≥1024
Catechin hydrate	1024	16	64	1024	1024	≥1024
Epigallocatechin	64	64	64	32	256	512
L-canavanine	>1024	128	128	512	≥1024	≥1024
Sulphathiazole	256	8	8	1024	512	≥1024
4-Nitro N-oxide	64	8	8	64	256	≥1024
p-Benzoquinone	8	64	64	128	1024	512
Esculin hydrate	512	256	256	512	1024	≥1024
Baicalein hydrate	4	512	512	64	1024	1024
Cinnamaldehyde	<1	256	256	<1	512	512
Farnesol	<1	128	128	<1	128	128

V1: Vancomycin+Curcumine, V2: Vancomycin +Azathioprine, V3: Vancomycin +Resvatrol, V4: Vancomycin+Catechin hydrate, V5: Vancomycin +Epigallocatechin, V6: Vancomycin+L-canavanine, V7: Vancomycin+Sulphathiazole, V8: Vancomycin+4-Nitro N-oxide, V9: Vancomycin+p-Benzoquinone, V10: Vancomycin+Esculin hydrate, V11: Vancomycin+Baicalein hydrate, V12: Vancomycin+Cinnamaldehyde, V13: Vancomycin+Farnesol.

CONCLUSIONS

- ✓ Combinations of sulfatiyazol, 4-nitropyridine N-oxit, esculin hydrate, cinnamaldehyde and farnesol with the vancomycin kills the *S.aureus* cells completely and increase the effect of vancomycin as an antibiofilm agent.
- ✓ Curcumin, azathioprine, resvatrol, epigallocatechin and baicalein hydrat have also additive effect on antibiofilm property of vancomycin.
- ✓ *S.aureus* cells in polymicrobial biofilm were more susceptible to vancomycin and vancomycin combinations of QSIs when compared with the cells in monomicrobial biofilm.

Table 2: Sessile cell counts of *S.aureus* ATCC 35556 in monomicrobial biofilms

<i>S.aureus</i> ATCC 35556	0,5	1	2	4	8	16	32
Vancomycin	5,5	6,2	6,6	-	-	-	-
V1	6,2	-	-	-	-	-	-
V2	2,7	-	-	-	-	-	-
V3	5,4	5,1	-	-	-	-	-
V4	2,6	2,9	3,1	3,7	3,5	3,3	3,7
V5	3,04	-	-	-	-	-	-
V6	3,5	2,7	4	3,6	4,3	4,3	4,3
V7	-	-	-	-	-	-	-
V8	-	-	-	-	-	-	-
V9	2,8	4,5	4,4	3,5	4,8	4,1	4,9
V10	-	-	-	-	-	-	-
V11	5,4	-	-	-	-	-	-
V12	-	-	-	-	-	-	-
V13	-	-	-	-	-	-	-

V1: Vancomycin+Curcumine, V2: Vancomycin +Azathioprine, V3: Vancomycin +Resvatrol, V4: Vancomycin+Catechin hydrate, V5: Vancomycin +Epigallocatechin, V6: Vancomycin+L-canavanine, V7: Vancomycin+Sulphathiazole, V8: Vancomycin+4-Nitro N-oxide, V9: Vancomycin+p-Benzoquinone, V10: Vancomycin+Esculin hydrate, V11: Vancomycin+Baicalein hydrate, V12: Vancomycin+Cinnamaldehyde, V13: Vancomycin +Farnesol, -: No growth

Table 4: Sessile cell counts of MRSA in monomicrobial biofilms

MRSA	0,0625	0,125	0,25	0,5	1	2
Vancomycin	0,62	0,4	0	0,5	0,4	0,9
V1	-	-	-	-	-	-
V2	2,9	3,2	3,5	3,8	3,9	2,8
V3	0,8	1	1	0,9	0,6	0,5
V4	1,2	1,4	0,9	0,9	1,3	1,3
V5	1,8	1,9	2,3	2,4	2,9	2,7
V6	0,8	0,5	0	0	0,5	1,2
V7	2,7	2,7	3,9	4,1	4,8	3,8
V8	1,2	0,9	0,9	0,8	0,6	0,5
V9	1	0,9	1,1	1,2	1,1	1,1
V10	1,6	1,9	1,9	1,9	1,8	1,5
V11	0,6	0,8	0,5	0,7	1	1,1
V12	-	-	-	-	-	-
V13	-	-	-	-	-	-

V1: Vancomycin+Curcumine, V2: Vancomycin +Azathioprine, V3: Vancomycin +Resvatrol, V4: Vancomycin+Catechin hydrate, V5: Vancomycin +Epigallocatechin, V6: Vancomycin+L-canavanine, V7: Vancomycin+Sulphathiazole, V8: Vancomycin+4-Nitro N-oxide, V9: Vancomycin+p-Benzoquinone, V10: Vancomycin+Esculin hydrate, V11: Vancomycin+Baicalein hydrate, V12: Vancomycin+Cinnamaldehyde, V13: Vancomycin +Farnesol, -: No growth

Table 3: Sessile cell counts of *S.aureus* ATCC 35556 in polymicrobial biofilms

<i>S.aureus</i> ATCC 35556	0,0625	0,125	0,25	0,5	1	2
Vancomycin	0,2	0,9	-	-	-	-
V1	1,3	2,6	2,6	2,6	2,6	2,6
V2	2,3	-	-	-	-	-
V3	0,3	1	1,4	2,6	2,6	-
V4	1,4	1,4	1,4	1,8	2,2	-
V5	0,8	1,1	2,3	-	-	-
V6	0,3	1,4	1,8	-	-	-
V7	0	0	0	0,2	2,65	-
V8	0	0	0,5	2,2	-	-
V9	0	0,1	0,9	2,65	-	-
V10	0	0	0	0	0	0,8
V11	0,3	1,3	1,6	1,6	1,6	1,8
V12	-	-	-	-	-	-
V13	-	-	-	-	-	-

V1: Vancomycin+Curcumine, V2: Vancomycin +Azathioprine, V3: Vancomycin +Resvatrol, V4: Vancomycin+Catechin hydrate, V5: Vancomycin +Epigallocatechin, V6: Vancomycin+L-canavanine, V7: Vancomycin+Sulphathiazole, V8: Vancomycin+4-Nitro N-oxide, V9: Vancomycin+p-Benzoquinone, V10: Vancomycin+Esculin hydrate, V11: Vancomycin+Baicalein hydrate, V12: Vancomycin+Cinnamaldehyde, V13: Vancomycin +Farnesol, -: No growth

Table 5: Sessile cell counts of MRSA in polymicrobial biofilms

MRSA	0,0625	0,125	0,25	0,5	1	2	4
Vancomycin	0	0	0	0	0	0	0,2
V1	1,7	1,8	-	-	-	-	-
V2	0,9	1	1,2	1,2	0,9	1,6	0
V3	0	0	0,3	0	1,1	0,6	0
V4	0	0	0	0	0	0	0
V5	0	0	0	0	0	0	0
V6	0	0	0	0	0	0,9	0
V7	0,5	0	0	0	0	0	0
V8	0	0	0	0	0,7	0,9	0
V9	0	0	0	0	0	1,2	0
V10	0	0	0	0	0	1,8	0
V11	0	0	0	0	0	0,7	0
V12	2,5	-	-	-	-	-	-
V13	-	-	-	-	-	-	-

V1: Vancomycin+Curcumine, V2: Vancomycin +Azathioprine, V3: Vancomycin +Resvatrol, V4: Vancomycin+Catechin hydrate, V5: Vancomycin +Epigallocatechin, V6: Vancomycin+L-canavanine, V7: Vancomycin+Sulphathiazole, V8: Vancomycin+4-Nitro N-oxide, V9: Vancomycin+p-Benzoquinone, V10: Vancomycin+Esculin hydrate, V11: Vancomycin+Baicalein hydrate, V12: Vancomycin+Cinnamaldehyde, V13: Vancomycin +Farnesol, -: No growth