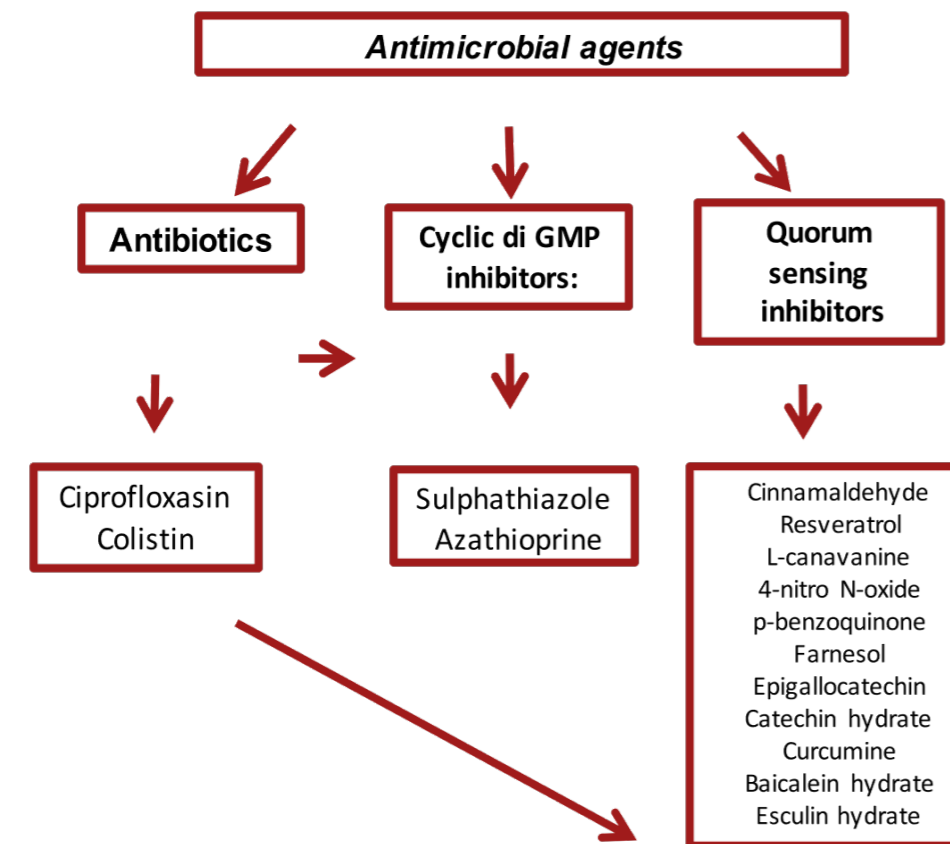




INTRODUCTION

Biofilms are frequently found in chronic and medical devices related infections and less susceptible to antimicrobial agents. Recent studies have demonstrated that biofilms are polymicrobial. 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. *Pseudomonas aeruginosa* is one of the major pathogen related with the biofilm infections and significant problem in hospital infections. In our study polymicrobial biofilms were reproducibly grown, consisting of *S.aureus*, *Paeruginosa*, *E.faecalis* and *C.albicans* in a 96 well microtiter plate. The antibiofilm effect of colistin and ciprofloxacin 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 *Paeruginosa*.

METHODS



Microorganisms:

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

- ✓ Monomicrobial biofilm models of *Paeruginosa* ATCC 47085 and a carbapenem resistance *Paeruginosa* isolate and polymicrobial biofilm models consisting of *C.albicans*, *E.faecium*, *Paeruginosa* 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 *Pa* 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 *P.aeruginosa* ATCC 47085 and carbapenem resistance *P.aeruginosa* isolate

	<i>P.aeruginosa</i> ATCC 47085			Carbapenem resistance <i>P.aeruginosa</i>		
	MIC	MBIC	MBEC	MIC	MBIC	MBEC
Ciprofloxacin/Colistin	<1	64	64	<1	32	64
C1*/C1*	-	≥128	≤0.0625	-	≥128	32
C2*/C2*	-	≤0.0625	0.25	-	≤0.0625	32
C3*/C3*	-	0.25	0.25	-	8	32
C4*/C4*	-	≤0.0625	0.5	-	16	16
C5*/C5*	-	128	>128	-	32	32
C6*/C6*	-	0.125	0.125	-	64	64
C7*/C7*	-	128	16	-	≤0.0625	32
C8*/C8*	-	0.25	25	-	8	16
C9*/C9*	-	0.125	0.5	-	8	16
C10*/C10*	-	≤0.0625	0.125	-	16	32
C11*/C11*	-	0.125	1	-	3	32
C12*/C12*	-	≤0.0625	≤0.0625	-	≤0.0625	≤0.0625
C13*/C13*	-	≤0.0625	8	-	≥128	4
Curcumine	512	256	512	1024	32	≥1024
Azathioprine	512	64	1024	512	64	≥1024
Resvatrol	256	≥1024	>1024	256	1024	≥1024
Catehin hydrate	512	1024	>1024	512	512	≥1024
Epigallocatechin	128	≥1024	>1024	128	1024	≥1024
L-canavanine	512	≥1024	>1024	512	512	≥1024
Sulphathiazole	128	≥1024	>1024	512	512	≥1024
4-Nitro N-oxide	64	≥1024	>1024	64	≥1024	≥1024
p-Benzoquinone	256	≥1024	>1024	256	512	≥1024
Esculin hydrate	256	512	>1024	256	128	≥1024
Baicalein hydrate	256	512	>1024	64	512	≥1024
Cinnamaldehyde	1024	512	1024	1024	256	512
Farnesol	1024	512	>1024	1024	≥1024	≥1024

C1: Ciprofloxacin+Curcumine, C2: Ciprofloxacin +Azathioprine, C3: Ciprofloxacin +Resvatrol, C4: Ciprofloxacin +Catechin hydrate, C5: Ciprofloxacin +Epigallocatechin, C6: Ciprofloxacin +L-canavanine, C7: Ciprofloxacin +Sulphathiazole, C8: Ciprofloxacin +4-Nitro N-oxide, C9: Ciprofloxacin +p-Benzoquinone, C10: Ciprofloxacin +Esculin hydrate, C11: Ciprofloxacin+Baicalein hydrate, C12: Ciprofloxacin+Cinnamaldehyde, C13: Ciprofloxacin +Farnesol. C1*: Colistin+Curcumine, C2*: Colistin+Azathioprine, C3*: Colistin+Resvatrol, C4*: Colistin+ Catechin hydrate, C5*: Colistin+Epigallocatechin, C6*: Colistin+L-canavanine, C7*: Colistin+Sulphathiazole, C8*: Colistin+4-Nitro N-oxide, C9*: Colistin+p-Benzoquinone, C10*: Colistin+ Esculin hydrate, C11*: Colistin+Baicalein hydrate, C12*: Colistin+Cinnamaldehyde, C13*: Colistin+Farnesol. *: Treated with colistin. *: Treated with ciprofloxacin. -: Non tested.

CONCLUSIONS

- ✓ Except for sulfathiazole and epigallocatechin, all QSIs increased the effect of ciprofloxacin as an antibiofilm agent.
- ✓ *Paeruginosa* ATCC 47085 in polymicrobial biofilm was less susceptible than carbapenem resistant isolate of *Paeruginosa* to the combinations of ciprofloxacin with the QSIs when compared with the cells in monomicrobial biofilms.

Table 2: *P.aeruginosa* ATCC 47085 sessile cell counts in monomicrobial biofilms

<i>P.aeruginosa</i> ATCC 47085	0,0625	0,125	0,25	0,5	1	2
Ciprofloxacin	3,4	3,8	4,6	4,9	5	5,7
C1	-	-	-	-	-	-
C2	6,1	-	-	-	-	-
C3	3,6	2,7	-	-	-	-
C4	5,1	5,4	6,6	-	-	-
C5	1,3	3,3	3,8	5,2	5,4	6,2
C6	5,2	-	-	-	-	-
C7	2,7	2,7	5,2	4,9	5,9	6,23
C8	4,1	-	-	-	-	-
C9	4	5,3	6,9	-	-	-
C10	6,5	-	-	-	-	-
C11	4,5	5,6	6,9	-	-	-
C12	-	-	-	-	-	-
C13	4,8	4,6	-	-	-	-

C1: Ciprofloxacin+Curcumine, C2: Ciprofloxacin +Azathioprine, C3: Ciprofloxacin +Resvatrol, C4: Ciprofloxacin +Catechin hydrate, C5: Ciprofloxacin +Epigallocatechin, C6: Ciprofloxacin +L-canavanine, C7: Ciprofloxacin +Sulphathiazole, C8: Ciprofloxacin +4-Nitro N-oxide, C9: Ciprofloxacin +p-Benzoquinone, C10: Ciprofloxacin +Esculin hydrate, C11: Ciprofloxacin+Baicalein hydrate, C12: Ciprofloxacin+Cinnamaldehyde, C13: Ciprofloxacin +Farnesol. -: No growth

Table 4: Carbapenem resistance *Paeruginosa* sessile cell counts in monomicrobial biofilms

<i>P.aeruginosa</i>	0,0625	0,125	0,25	0,5	1	2	4	8
Colistin	0	0	0	0	0,8	0,8	0,9	1
C1	0,1	1,7	4,1	3,3	3,5	4,8	6,3	5,2
C2	0	0	0,7	0,3	1,8	3	3,7	5,6
C3	0	0	0,6	0,8	0,1	0,7	0,3	1,3
C4	0	0	0,5	0,8	0,2	0,2	0,4	0,1
C5	0	0	0	1,1	0,4	0,4	0,2	3,9
C6	0	0	0	0,7	0,1	0,1	0,1	1,5
C7	0	0,2	0,1	0,2	0,3	0,3	1	1,3
C8	0	0,3	0,2	0,6	0,7	1,8	3,1	3,7
C9	0	0,7	1	1,8	2,4	2,7	3,1	5,5
C10	0	0	0	0	0	0	0	0,4
C11	0	0	0,6	0,9	0,9	1,4	1,7	1,9
C12	-	-	-	-	-	-	-	-
C13	3,8	3,9	4	4,1	3,8	3,1	5,6	-

Colistin+Curcumine, C2: Colistin+ Azathioprine, C3: Colistin+Resvatrol, C4: Colistin+ Catechin hydrate, C5: Colistin+Epigallocatechin, C6: Colistin+L-canavanine, C7: Colistin+Sulphathiazole, C8: Colistin+4-Nitro N-oxide, C9: Colistin+p-Benzoquinone, C10: Colistin+ Esculin hydrate, C11: Colistin+Baicalein hydrate, C12: Colistin+Cinnamaldehyde, C13: Colistin+Farnesol. -:No growth.

Table 3: *P.aeruginosa* ATCC 47085 sessile cell counts in polymicrobial biofilms

<i>P.aeruginosa</i> ATCC 47085	0,0625	0,125	0,25	0,5	1	2
Ciprofloxacin	1	3,7	3,8	3,5	4,5	4,3
C1	3,8	4	4,4	4,5	4,6	4,9
C2	3,9	3,9	3,7	3,9	5,4	5,2
C3	0	5,4	4,6	3,6	3,9	4,7
C4	4,1	5,5	4,1	3,7	3,6	5,4
C5	0	2,8	2,6	3,5	3,5	4,7
C6	2,7	5,3	3,9	2,5	4,7	6
C7	2,4	4,6	3,3	4,8	4,4	4,7
C8	1,8	3,5	2,2	1,5	3,6	4,4
C9	0	3,6	3,1	3,9	4,5	3,9
C10	0	5,4	4,3	4	4,5	-
C11	0	3,8	3,7	2,4	3,5	4,6
C12	-	-	-	-	-	-
C13	3,1	2,6	3,3	3,3	2,8	3,4

C1: Ciprofloxacin+Curcumine, C2: Ciprofloxacin +Azathioprine, C3: Ciprofloxacin +Resvatrol, C4: Ciprofloxacin +Catechin hydrate, C5: Ciprofloxacin +Epigallocatechin, C6: Ciprofloxacin +L-canavanine, C7: Ciprofloxacin +Sulphathiazole, C8: Ciprofloxacin +4-Nitro N-oxide, C9: Ciprofloxacin +p-Benzoquinone, C10: Ciprofloxacin +Esculin hydrate, C11: Ciprofloxacin+Baicalein hydrate, C12: Ciprofloxacin+Cinnamaldehyde, C13: Ciprofloxacin +Farnesol. -: No growth

Table 5: Carbapenem resistance *Paeruginosa* sessile cell counts in polymicrobial biofilms

<i>P.aeruginosa</i>	0,125	0,25	0,5	1	2	4	8
Colistin	0	0	0	0	0	0	0
C1	3,1	3,8	4,8	-	-	-	-
C2	2,6	5,4	5,4	4,9	6,5	7,1	6,8
C3	2,9	3,1	3,4	3,4	5,2	6,1	4,2
C4	3,4	4,8	5,8	6,5	6,1	6,5	5,6
C5	2,4	3,9	5,4	5,3	6,4	6,3	5,8
C6	0	0	0	1,1	1,6	3,7	3,3
C7	3,4	3,4	3	2,7	2,5	6,8	-
C8	0	0	0	0	0	0	0
C9	0,8	2,9	3,7	3,7	5,6	2,8	4,7
C10	2,9	3,4	5,7	3,3	5,6	5,6	5,7
C11	0	0	0	0	0	0	0
C12	6,9	-	-	-	-	-	-
C13	-	-	-	-	-	-	-

Colistin+Curcumine, C2: Colistin+ Azathioprine, C3: Colistin+Resvatrol, C4: Colistin+ Catechin hydrate, C5: Colistin+Epigallocatechin, C6: Colistin+L-canavanine, C7: Colistin+Sulphathiazole, C8: Colistin+4-Nitro N-oxide, C9: Colistin+p-Benzoquinone, C10: Colistin+ Esculin hydrate, C11: Colistin+Baicalein hydrate, C12: Colistin+Cinnamaldehyde, C13: Colistin+Farnesol. -:No growth.