ORIGINAL RESEARCH - **Supplementary data**

Different effects of amniotic membrane homogenate on bacterial growth

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Different effects of amniotic membrane homogenate on the growth of uropathogenic *Escherichia coli*, *Staphylococcus aureus* and *Serratia marcescens*

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Supplementary data

Supplementary Table 1 Complete results of one-way ANOVA analysis that compared the effect of saline (0.9% NaCl), PBS and different dilutions of AM homogenate (undiluted H1, 2-fold diluted H2, 4-fold diluted H3) on bacterial growth. The values in green represent statistically significant data ($p < 0.05$).

Abbreviations: H1, undiluted AM homogenate; H2, 2-fold diluted AM homogenate; H3, 4-fold diluted AM homogenate.

Note: The statistically significant values for UPEC at t=0 h are visible in the case of added gentamicin, which had an immediate effect on the bacterial cells and thus on CFU/ml.

t [h]	Pairwise comparison	UPEC p-value	*S. aureus* p-value	*S. marcescens* p-value
0	0.9 % NaCl vs. gentamicin	**0,0067**	0,41	0,6735
0	0.9 % NaCl vs. H1	0,9585	0,9902	0,9935
0	0.9 % NaCl vs. H2	0,9018	0,9943	0,981
0	0.9 % NaCl vs. H3	> 0,9999	0,9346	0,9586
0	0.9 % NaCl vs. PBS	0,3824	> 0,9999	0,9913
0	H1 vs. gentamicin	**0,0132**	0,1824	0,8407
0	H1 vs. H2	0,9998	0,8704	> 0,9999
0	H1 vs. H3	0,9908	0,6694	0,9988
0	H2 vs. gentamicin	**0,03**	0,6983	0,9067
0	H2 vs. H3	0,9641	0,9984	> 0,9999
0	H3 vs. gentamicin	**0,0104**	0,8903	0,9714
0	PBS vs. gentamicin	0,1279	0,4176	0,8586
0	PBS vs. H1	0,7522	0,989	> 0,9999
0	PBS vs. H2	0,9101	0,995	> 0,9999
0	PBS vs. H3	0,5162	0,9387	0,9993
1	0.9 % NaCl vs. gentamicin	**< 0,0001**	**< 0,0001**	**< 0,0001**
1	0.9 % NaCl vs. H1	0,3987	**0,0021**	0,9237
1	0.9 % NaCl vs. H2	0,4603	**0,0015**	0,9707
1	0.9 % NaCl vs. H3	0,6601	**0,0062**	0,781
1	0.9 % NaCl vs. PBS	0,957	0,9988	> 0,9999
1	H1 vs. gentamicin	**< 0,0001**	**< 0,0001**	**< 0,0001**
1	H1 vs. H2	> 0,9999	0,9999	0,9998
1	H1 vs. H3	0,9997	0,9833	0,9967
1	H2 vs. gentamicin	**< 0,0001**	**< 0,0001**	**< 0,0001**
1	H2 vs. H3	0,9999	0,9401	0,9795
1	H3 vs. gentamicin	**< 0,0001**	**< 0,0001**	**< 0,0001**
1	PBS vs. gentamicin	**< 0,0001**	**< 0,0001**	**< 0,0001**
1	PBS vs. H1	**0,0467**	**0,0039**	0,774
1	PBS vs. H2	0,0706	**0,0026**	0,8874
1	PBS vs. H3	0,1707	**0,0115**	0,5641

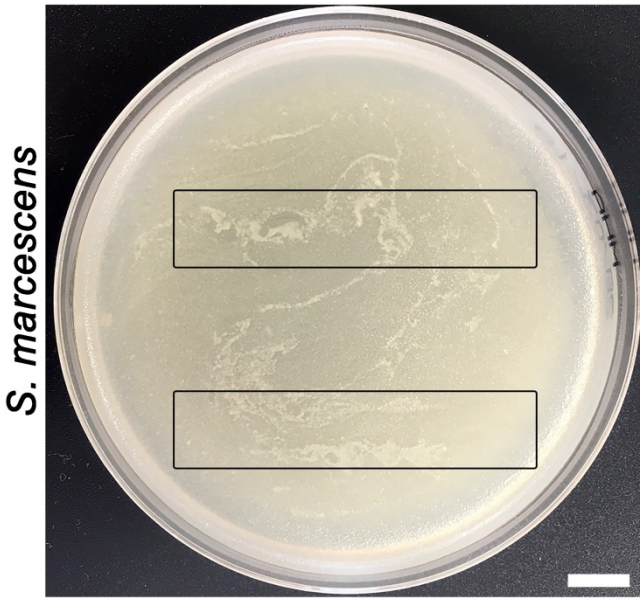
t [h]	Pairwise comparison	UPEC p-value	*S. aureus* p-value	*S. marcescens* p-value
2	0.9 % NaCl vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
2	0.9 % NaCl vs. H1	< 0,0001	< 0,0001	0,9916
2	0.9 % NaCl vs. H2	< 0,0001	< 0,0001	0,7517
2	0.9 % NaCl vs. H3	< 0,0001	< 0,0001	0,4512
2	0.9 % NaCl vs. PBS	> 0,9999	0,339	0,9858
2	H1 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
2	H1 vs. H2	0,9973	0,9998	0,9228
2	H1 vs. H3	0,9986	> 0,9999	0,6101
2	H2 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
2	H2 vs. H3	0,9697	0,997	0,9772
2	H3 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
2	PBS vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
2	PBS vs. H1	< 0,0001	0,0016	0,6814
2	PBS vs. H2	< 0,0001	0,0011	0,2071
2	PBS vs. H3	< 0,0001	0,0022	0,0861
3	0.9 % NaCl vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
3	0.9 % NaCl vs. H1	< 0,0001	< 0,0001	0,5422
3	0.9 % NaCl vs. H2	< 0,0001	< 0,0001	0,8041
3	0.9 % NaCl vs. H3	< 0,0001	< 0,0001	0,7934
3	0.9 % NaCl vs. PBS	> 0,9999	0,9999	0,9857
3	H1 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
3	H1 vs. H2	0,9997	0,9997	0,9915
3	H1 vs. H3	0,9982	0,9807	0,9978
3	H2 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
3	H2 vs. H3	> 0,9999	0,9227	> 0,9999
3	H3 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
3	PBS vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
3	PBS vs. H1	< 0,0001	< 0,0001	0,0993
3	PBS vs. H2	< 0,0001	< 0,0001	0,2492
3	PBS vs. H3	< 0,0001	< 0,0001	0,276
4	0.9 % NaCl vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
4	0.9 % NaCl vs. H1	< 0,0001	< 0,0001	0,8774
4	0.9 % NaCl vs. H2	< 0,0001	< 0,0001	0,9686
4	0.9 % NaCl vs. H3	< 0,0001	< 0,0001	0,9976
4	0.9 % NaCl vs. PBS	0,9928	0,9998	0,9998
4	H1 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
4	H1 vs. H2	> 0,9999	> 0,9999	0,9985
4	H1 vs. H3	0,9997	0,7943	0,9767
4	H2 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
4	H2 vs. H3	> 0,9999	0,8093	0,9991
4	H3 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
4	PBS vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
4	PBS vs. H1	< 0,0001	< 0,0001	0,5798
4	PBS vs. H2	< 0,0001	< 0,0001	0,8009
4	PBS vs. H3	< 0,0001	< 0,0001	0,9598

t [h]	Pairwise comparison	UPEC p-value	*S. aureus* p-value	*S. marcescens* p-value
5	0.9 % NaCl vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
5	0.9 % NaCl vs. H1	< 0,0001	< 0,0001	0,0015
5	0.9 % NaCl vs. H2	< 0,0001	< 0,0001	0,0546
5	0.9 % NaCl vs. H3	< 0,0001	< 0,0001	0,0405
5	0.9 % NaCl vs. PBS	> 0,9999	0,9996	0,9988
5	H1 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
5	H1 vs. H2	> 0,9999	0,9995	0,2105
5	H1 vs. H3	> 0,9999	0,9	0,4654
5	H2 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
5	H2 vs. H3	> 0,9999	0,9757	0,9985
5	H3 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
5	PBS vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
5	PBS vs. H1	< 0,0001	< 0,0001	< 0,0001
5	PBS vs. H2	< 0,0001	< 0,0001	0,0063
5	PBS vs. H3	< 0,0001	< 0,0001	0,0055
6	0.9 % NaCl vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
6	0.9 % NaCl vs. H1	< 0,0001	0,0001	0,0859
6	0.9 % NaCl vs. H2	< 0,0001	< 0,0001	0,1429
6	0.9 % NaCl vs. H3	< 0,0001	< 0,0001	0,0891
6	0.9 % NaCl vs. PBS	> 0,9999	0,9989	0,965
6	H1 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
6	H1 vs. H2	0,9995	0,8687	0,9989
6	H1 vs. H3	0,9814	0,1999	> 0,9999
6	H2 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
6	H2 vs. H3	0,9326	0,7343	0,9954
6	H3 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
6	PBS vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
6	PBS vs. H1	< 0,0001	0,0002	0,1422
6	PBS vs. H2	< 0,0001	< 0,0001	0,2534
6	PBS vs. H3	< 0,0001	< 0,0001	0,1522
7	0.9 % NaCl vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
7	0.9 % NaCl vs. H1	< 0,0001	0,0006	0,9954
7	0.9 % NaCl vs. H2	< 0,0001	0,0002	0,9979
7	0.9 % NaCl vs. H3	< 0,0001	< 0,0001	0,9957
7	0.9 % NaCl vs. PBS	0,9785	> 0,9999	0,9988
7	H1 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
7	H1 vs. H2	0,8573	0,9863	> 0,9999
7	H1 vs. H3	> 0,9999	0,2397	0,8514
7	H2 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
7	H2 vs. H3	0,8452	0,5329	0,8871
7	H3 vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
7	PBS vs. gentamicin	< 0,0001	< 0,0001	< 0,0001
7	PBS vs. H1	< 0,0001	0,0005	> 0,9999
7	PBS vs. H2	< 0,0001	0,0002	> 0,9999
7	PBS vs. H3	< 0,0001	< 0,0001	0,9051

Supplementary Table 2 Complete results of one-way ANOVA analysis that compared the growth of *S. marcescens* in Mueller-Hinton broth, undiluted AM homogenate, 2- fold and 4-fold diluted AM homogenate and PBS. The values in green represent statistically significant data ($p<0.05$).

Abbreviations: MHM, Mueller-Hinton broth; H1, undiluted AM homogenate; H2, 2-fold diluted AM homogenate; H3, 4-fold diluted AM homogenate.

t [h]	Pairwise comparison	p-value
0	MHM vs. H1	0,9978
0	MHM vs. H2	> 0,9999
0	MHM vs. H3	0,9974
0	MHM vs. PBS	0,9000
0	H1 vs. H2	0,9998
0	H1 vs. H3	0,9675
0	H1 vs. PBS	0,7777
0	H2 vs. H3	0,9903
0	H2 vs. PBS	0,8529
0	H3 vs. PBS	0,9729
7	MHM vs. H1	0,2953
7	MHM vs. H2	**0,0029**
7	MHM vs. H3	**0,0049**
7	MHM vs. PBS	**< 0,0001**
7	H1 vs. H2	0,1695
7	H1 vs. H3	0,2552
7	H1 vs. PBS	**0,0004**
7	H2 vs. H3	0,9991
7	H2 vs. PBS	**0,0311**
7	H3 vs. PBS	**0,0199**



Supplementary Figure 1 A clinical strain of *S. marcescens* was tested to observe, if its growth is at all inhibited by AM homogenate (H1, its preparation is described in Materials and Methods, chapter Amniotic membrane homogenate preparation). No zones of inhibition were detected, suggesting that the resistance against growth inhibitory compounds of AM is species-specific. Muller-Hinton soft agar was first cooked at 100°C, cooled to 48°C, inoculated with 100 µl of overnight culture of *S. marcescens* and poured over the Muller-Hinton agar plate. After 15 minutes of incubation at room temperature, 3-times of 5 µl (top row) and 3-times of 10 µl (bottom row) of AM homogenates were placed on the agar plate and incubated at 37°C for 24 hours. Frames mark positions of AM homogenates. Scale bar: 10 mm.