Antimicrobial activity of formulas developed by Trulstech Innovation KB on behalf of Biomimetic Technology Ltd

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Abbreviations

- WHO: World Health Organization
- EUCAST: European Committee on Antimicrobial Susceptibility Testing
- LB Broth: Luria-Bertani Broth (Culture medium for bacteria)
- PBS: Phosphate Buffered Saline (Dilution/assay buffer)
- *S. aureus*: *Staphylococcus aureus* (gram positive bacterium)
- MRSA: Methicillin-resistant *S. aureus* (resistance against penicillins)
- *E. coli*: *Escherichia coli* (gram negative bacterium)'
- ESBL: Extended Spectrum Beta Lactamase (resistance against penicillins)
- MIC: Minimum Inhibitory Concentration (Lowest concentration/amount of test substance that inhibits bacterial growth)
- MBC: Minimum Bactericidal Concentration (Lowest concentration/amount of test substance that kills >99.9 of the bacteria)

Introduction

S. aureus is a gram-positive bacterium and one of the most common skin-colonizing bacteria of humans. This bacterium has acquired resistance against a wide range of different antibiotics and has been highlighted by WHO as one of the priority pathogens for which new antimicrobials are urgently needed. Methicillin-resistant *S. aureus* (MRSA) is often identified to be multi-drug resistant and can cause superficial skin and soft tissue infections, and life-threatening infections, such as sepsis.

E. coli is a gram-negative bacterium and part of the normal microflora that is often found in the lower intestines of humans. This bacterium is used as an indicator microorganism to investigate possible fecal contamination of environmental samples, such as water and food. Some highly virulent, disease-causing strains of *E. coli* have been identified in humans, including ESBL-producing strains, that can cause several diseases, of which diarrhoea is the most common consequence that particularly affects children and elderly, causing more than 420 000 deaths every year.

The aim of this study was to determine the antimicrobial activity of innovative and biodegradable test solutions that were developed by Trulstech Innovation KB, on behalf of Biomimetic Technology Ltd, provided by Jimmy Lundström, CEO of JL Kemi AB, against *S. aureus* and *E. coli*.

The rationale behind assessing the antimicrobial activity of the test solutions against *S. aureus* and *E. coli* is the differences in structure between these bacteria (Figure 1). Gram-negative bacteria (*E. coli*) have an inner and outer phospholipid membrane with a thin peptidoglycan layer (cell wall) in between. Gram-positive bacteria (*S. aureus*) have one phospholipid membrane that is covered by a thick cell wall composed of multiple layers of peptidoglycan. This cell wall can become even thicker and contribute to heterogenous antibiotic resistance, i.e., it is more difficult for cell wall synthesis inhibitors, such as penicillin, to penetrate and reach its target.

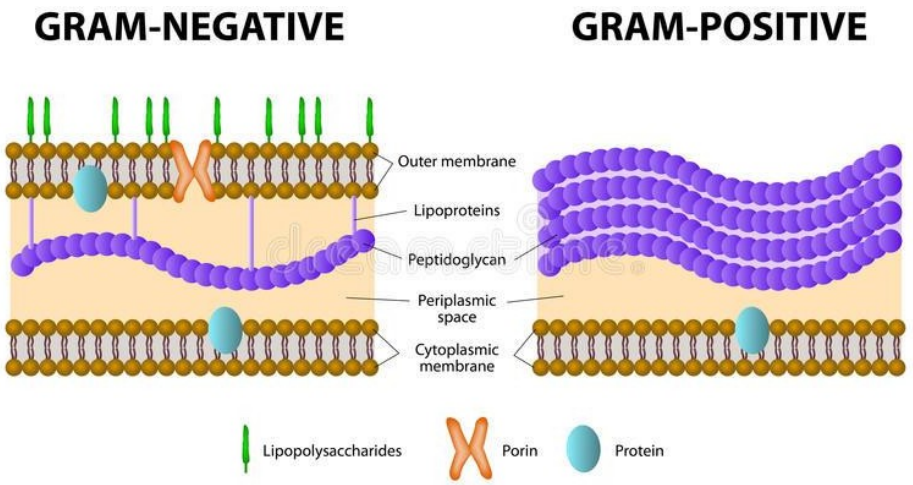

Figure 1. Simplified figure showing the differences in structure between gram-negative and gram-positive bacteria.

Method

The broth microdilution method was used to assess the antimicrobial activity of the test solutions by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to EUCAST (www.eucast.org), and Clinical and Laboratory Standards Institute (www.clsi.org). Briefly, the bacteria were cultured onto Luria-Bertani (LB) agar plates and incubated overnight at 37 ˚C. A single colony was inoculated into 5 ml of LB broth and the bacteria were allowed to growth overnight at 37 ˚C on an orbital shaker (400 RPM). Bacterial concentration was adjusted to correspond to 10^9 CFU/ml, which was determined by viable count.

Two-fold serial dilutions of the test solutions were prepared in 96-well plates using phosphate buffeted saline (PBS) (Figure 2). The bacteria were diluted in LB broth and 100 µl (5×10^5 CFU/ml) of bacterial suspension was added to all the wells, followed by incubation at 37 ˚C on a shaker (400 RPM) for 20 h. Visual inspection and spectroscopical quantification (620 nm) was used to determine the MIC as the lowest concentration that completely inhibited bacterial growth. A sample of 10 µl from all the inhibited wells was pipetted onto LB agar plates followed by incubation overnight at 37 ˚C. MBC was determined as the lowest concentration where no growth of bacterial colonies was observed on the plates.

A

	1	2	3	4	5	6	7	8	9	10	11	12
A	50	25	12.5	6.3	3.1	1.6	0.8	0.4	0.2	0.1	0.05	0.025
B	0.013	0.0063	0.0031	0.0016	0.00078	0.00039						
C												
D												
E												
F												
G												
H												

B

	1	2	3	4	5	6	7	8	9	10	11	12
A	2	4	8	16	32	64	128	256	512	1024	2048	4096
B	8192	16384	32786	65536	131072	262144						
C												
D												
E												
F												
G												
H												

Figure 2. Schematic representation of the serially diluted test solutions is presented in **A-** as percent (%), e.g., 50%, of the total volume in well A1, and **B-** as the number of times diluted (X), e.g., 2X.

Results

The results for each test solution are presented in two different graphs (X-times diluted and as %) showing the lowest amount of test solution that is required to suppress bacterial growth (bacteriostatic effect, MIC). TT51 was shown to be a potent antimicrobial test solution in which a final amount of 12.5% of the total volume (or when the solution is diluted 8 times) resulted in complete bacterial inhibition of both *S. aureus* and *E. coli* (Figure 3A-B). Test solution TT51 V2 was even more potent and a final content of 1.5 %-3.1 % (32X-64X) was sufficient to suppress the growth of all four indicator strains (Figure 3C-D).

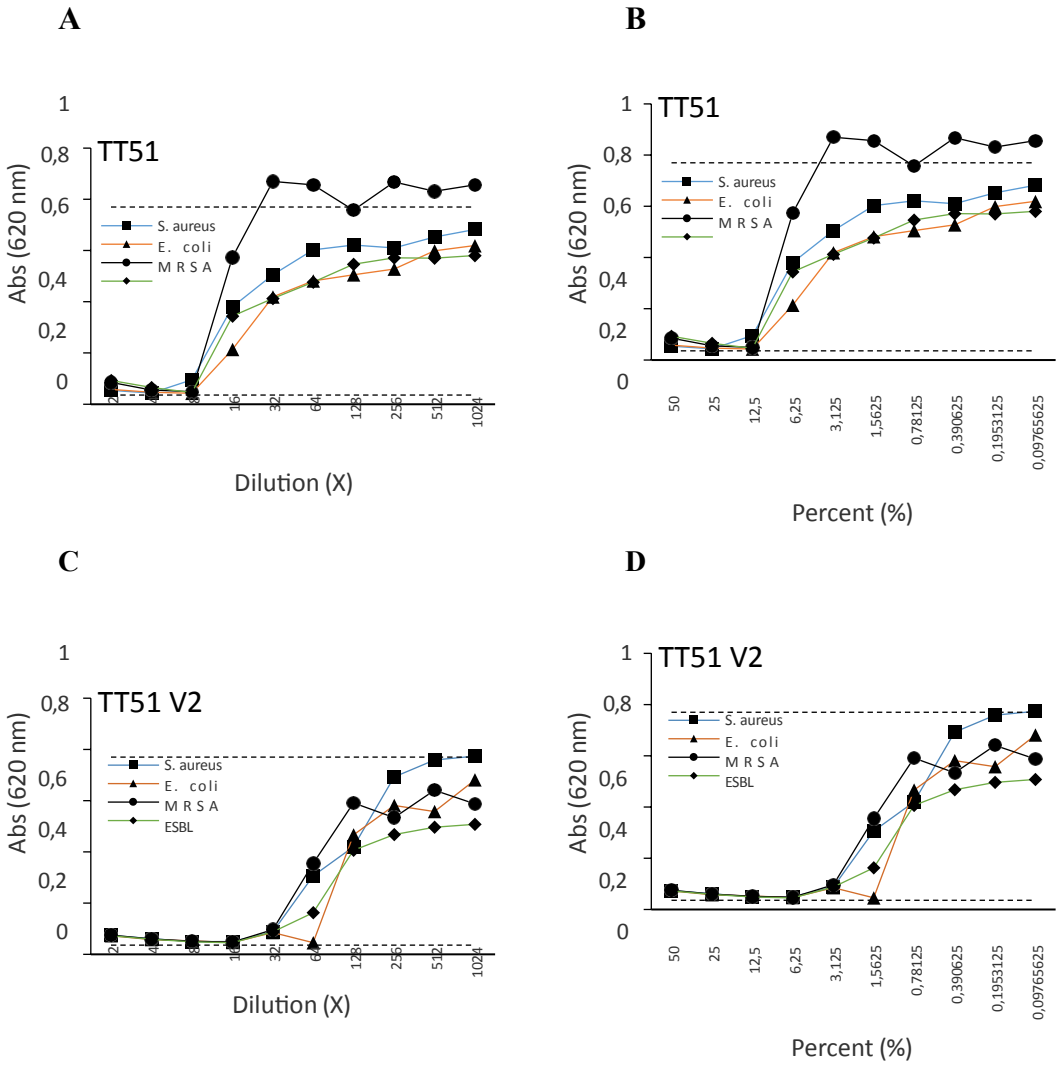

Figure 3. Bacterial inhibition (bacteriostatic effect, MIC) of the test solutions TT51 (**A-B**) and TT51 V2 (**C-D**) against *S. aureus*, *E. coli*, MRSA, and ESBL is presented as the number of times diluted (X) and as percent (%). Test solutions TT51 and TT51 V2 showed potent inhibitory effects against the test bacteria. Absorbance was measured at 620 nm after 20 h incubation of the bacteria with the test solutions.

Inhibition of bacterial growth (MIC) is an important indicator of the antimicrobial activity of test solutions, however this endpoint does not provide data of bacterial elimination (bactericidal effect, MBC). Samples from all the inhibited wells by test solutions TT51and TT51 V2 were cultured on agar plates to investigate the bactericidal effects. The results show that although test solution TT51 can be diluted up to 8X and most potently suppress *S. aureus* growth, a higher concentration is required (4X) to completely eliminate the bacteria (Table 1). *E. coli* was more susceptible to test solution TT51, in which a final dilution of 8X (or 12.5 % of the total volume) was both bacteriostatic and bactericidal. TT51 V2 showed to be a potent bactericidal

solution, in which a final dilution of 32X (3.1 %) eliminated *E. coli*, MRSA, and ESBL, while *S. aureus* required 16X (6.3 %).

Table 1. Summary of the antimicrobial activity of test solutions TT51 and TT51 V2 showing the bacteriostatic (MIC) and bactericidal (MBC) activity against *S. aureus*, *E. coli*, MRSA, and ESBL The results are presented as the lowest concentration/amount of test solution that inhibits the growth (MIC) and eliminates (MBC) the bacteria as the number of times diluted (X).

	S. aureus		***E. coli***		**MRSA**		**ESBL**	
Test solution	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
TT-51	8X	4X	8X	8X	8X	4X	8X	8X
TT-51 V2	32X	16X	64X	32X	32X	32X	32X	32X

Conclusion

Test solution TT51 and TT51 V2 have potent broad-spectrum antimicrobial characteristics that adds another dimension to its commercial applications, e.g., hospitals and clinical settings as a detergent and disinfectant agent. TT51 can be diluted up to eight times (8X), and TT51 V2 up to 32X, and still retain their bacteriostatic and bactericidal activities against antibiotic sensitive strains and multidrug-resistant strains. These effects have been validated in laboratory settings at Örebro University using standard *in vitro* methods.