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# The prevalence of antimicrobial-producing Gram-positive bacteria in human gut: a preliminary study

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## Abstract

**Background:** Human gut microbiome is an excellent source for searching novel antimicrobials which is currently in need due to the raise of drug resistance bacteria. Many Gram-positive bacteria isolated from human gut have been reported to produce antimicrobial compounds but still only few studies investigating the prevalence of these bacteria in human gut.

**Methods:** We took stool samples from 19 adult participants (age: 20–70 years; ethnicity: European and Asian). Stool samples obtained from 7 females and 12 males. We screened for Gram-positive antimicrobial-producing bacteria from the stool samples and identified the positive ones using 16s rRNA sequencing.

**Results:** Here, we reported that antimicrobial-producing Gram-positive bacteria can be found in the stool samples of 6 out of 19 participants. By screening against *Staphylococcus aureus* USA300 and *Pseudomonas aeruginosa* PAO1, some isolates exhibited a different inhibition activity compared to the previously reported antimicrobial compounds.

**Conclusion:** Our findings showed that some strains isolated from human gut exhibits a novel antimicrobial activity which implies that there could still be novel antimicrobial compounds in human gut produced by Gram-positive bacteria.

**Keywords:**

Antimicrobial-producing bacteria; Gram-positive bacteria; Gut microbiota; Microorganism



## Introduction

Human gut microbiome, due to its complexity, its pivotal contribution on human physiology and development [1-3], and its unravelled mystery, is becoming a hot spot for researcher to mine the resource for solving problems that human is facing. Particular problem that is arising is the need of novel antimicrobial to cope with the drug resistance bacteria, which become a major threat. Human gut microbiome can be an excellent source for researcher to search for novel antimicrobial compounds as it also plays a crucial role in protection against pathogen invasion [4,5], particularly in gastrointestinal tract [6-8].

There are many Gram-positive bacteria isolated from human gut have been reported to produce antimicrobial compounds, namely *Bacillus*, *Ruminococcus*, *Enterococcus*, *Lactobacillus*, etc [9]. However, there is still few studies investigating the prevalence of antimicrobial-producing Gram-positive bacteria in human gut. In this study we investigated the prevalence of these bacteria and identified them using 16S rRNA.

## Methods

### Stool Samples Collection

The use of human stool samples was approved by the Ethics Commission of the University of Tuebingen (approval no. 320/2017BO2). Stool samples were obtained from 19 adult participants (age: 20–70 years; ethnicity: European and Asian; location: Tuebingen and Stuttgart, Germany). Stool samples obtained from 7 female and 12 male participants.

### Screening for Antimicrobial Activity

The obtained stool samples were diluted and spread on modified SK medium with lower sodium azide (0.25%) [10]. After incubation at 37°C for 48 h, the obtained colonies were randomly picked and cultured in TSB medium in 96-well plates at 37°C for 24 hr. The supernatants were then harvested, and we used 100 µl of the supernatant to be mixed with 100 µl of freshly inoculated TSB medium at OD<sub>600</sub> 0.01 with *Staphylococcus aureus* USA300 or *Pseudomonas aeruginosa* PAO1 that has been grown as a preculture one day before. The mixture was carried out in a 96-well plate and incubated for 24 h at 37°C. OD<sub>600</sub> of each mixture were then measured to observe the growth of *S. aureus* USA300 or *P. aeruginosa* PAO1.

### Bacterial Isolates Identification

The isolates that showed an inhibition toward *S. aureus* USA300 or *P. aeruginosa* PAO1 were identified using 16s rRNA genes using 27F and 1392R primers [11-13]. The corresponding sequences were then analysed using blastn (NCBI).

## Results

We have examined the stool samples collected from 19 participants by performing the screening of the antimicrobial activity of the grown isolates in modified SK agar medium. The SK agar medium was purposely formulated as a staphylococci selective medium. We lowered the sodium azide concentration and successfully used it to grow Gram-positive bacteria on the agar. We picked 100 colonies of each participant carried out the screening (Fig. 1A). We observed there were 23 isolates that showed the growth inhibition activity against *S. aureus* USA300 and only 16 of them showed the growth inhibition activity towards *P. aeruginosa* PAO1 as well. We did not find any isolate that showed growth inhibition activity towards *P. aeruginosa* PAO1 only (Table 1).

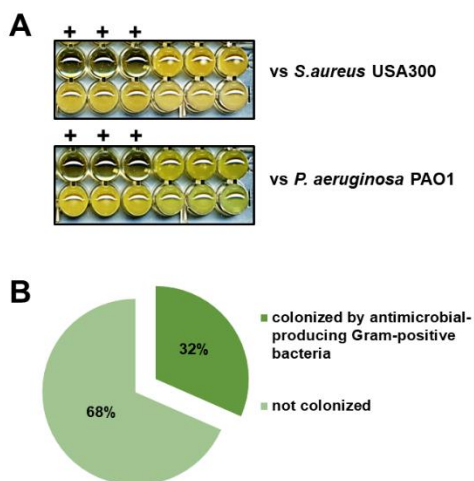
Participant	Isolate code	Growth inhibition activity		Species
		<i>S. aureus</i> USA300	<i>P. aeruginosa</i> PAO1	
5	EA7	+	-	<i>Corynebacterium aurimucosum</i>
6	FC5	+	+	<i>Enterococcus avium</i>
	FA5	+	+	<i>Enterococcus pallens</i>
	FG5	+	-	<i>Enterococcus avium</i>
	FH9	+	-	<i>Enterococcus xiangfangensis</i>
	FB5	+	-	<i>Enterococcus avium</i>
9	IC9	+	+	<i>Streptococcus tigurinus</i>
	IA10	+	+	<i>Streptococcus tigurinus</i>
	IC11	+	+	<i>Streptococcus tigurinus</i>
	IC10	+	+	<i>Streptococcus oralis</i>
	IE9	+	+	<i>Streptococcus tigurinus</i>
13	MD12	+	+	<i>Weissellaviridescens</i>
	MA9	+	+	<i>Weissellaviridescens</i>
	MC12	+	-	<i>Weissellaviridescens</i>
	MH7	+	-	<i>Weissellaviridescens</i>
	MG1	+	-	<i>Weissellaviridescens</i>
	MB12	+	-	<i>Weissellaviridescens</i>
16	PG1	+	+	<i>Enterococcus casseliflavus</i>
	PA1	+	-	<i>Enterococcus gallinarum</i>
	PH1	+	+	<i>Enterococcus gallinarum</i>
	PF1	+	+	<i>Enterococcus gallinarum</i>
21	UE9	+	+	<i>Streptococcus mitis</i>
	UC5	+	-	<i>Bacillus paralicheniformis</i>

+: there is growth inhibition activity; -: no inhibition activity observed

**Table 1:** Growth inhibition activity and species identification of positive isolates.

Out of the 19 participants, only 6 of them had bacteria that produced antimicrobial substances, and these bacteria were isolated from those individuals. This means that less than half of the participants were colonized by antimicrobial-producing Gram-positive bacteria, as shown in Figure 1B. Among 6 of the antimicrobial-producing Gram-positive bacteria-

harboring participants, only 5 of them were colonized by isolates that shown growth inhibition activity towards *P. aeruginosa* PAO1 (26%). The species identification of the 23 isolates exhibited that the positively shown antimicrobial activity are from genera that have been previously reported to produce certain antibacterial compound, namely *Streptococcus*, *Bacillus*, *Corynebacterium*, *Enterococcus*, and *Weissella* (Table 1).



**Figure 1:** Screening of antimicrobial activity, with the antimicrobial-producing isolates indicated with “+” (A) and Prevalence of antimicrobial-producing Gram-positive bacteria in sampled population (B).

## Discussion

The fact that there are bacteria that are able to produce antimicrobial compound as a gut commensal support the findings that one of the main functions of the good bacteria as a part of the gut microbiome is to prevent the pathogenic bacteria to colonize the human gut and control their population [14-16]. With the antimicrobial compound produced, these bacteria play a key role in maintaining the gut microbiome stability, resistance, and resilience against the pathogenic bacteria invasion. This implies that these bacteria crucially participate in keeping the gut health [17-19].

In this study, we identified some antimicrobial-producing bacteria from various genera. In genera *Streptococcus*, we identified 3 species: *S. tigurinus*, *S. mitis*, and *S. oralis*. All streptococcal isolates exhibited growth inhibition activity against both *S. aureus* USA300 and *P. aeruginosa* PAO1. The possible antimicrobial compound produced by these isolates is viridins. Viridins were reported to be produced by streptococci, particularly *Streptococcus sanguis* and *S. mitis*. This compound is active against Gram-negative and Gram-positive bacteria [20], which is in agreement with our results that streptococcal isolates showed

inhibition activity against both *S. aureus* USA300 and *P. aeruginosa* PAO1.

For genera *Bacillus*, *Corynebacterium*, and *Weissella*, we only detected 1 species for each: *B. paralicheniformis*, *C. aurimucosum*, and *W. viridescens*. *B. paralicheniformis* has been reported to be able to produce formicin, a lantibiotic that active against Gram-positive bacteria [21], similar phenotype shown by UC5 isolate. *C. aurimucosum* has not been specifically reported to produce any antimicrobial compound. However, some studies reported *Corynebacterium hydrocarboclastus* produces corynecin, a chloramphenicol analog that is active against Gram-positive and Gram-negative bacteria [22,23]. *Weissella*, a lactic acid bacterium, particularly species *W. confusa* is known to produce some bacteriocins called weissellicin and reported to active inhibiting the growth of namely *Bacillus cereus*, *Escherichia coli*, *P. aeruginosa* and *Micrococcus luteus* [24]. However, there can be a novel bacteriocin produced, since the spectrum for isolate MC12, MH7, MG1 and MB12 are only active against *S. aureus* USA300.

Enterococcal bacteria have been known to produce bacteriocins, such as enterocins, that are active against Gram-positive bacteria [25-28]. We observed similar activity for isolate FG5, FH9, and FB5 but not for FC5 and FA5, which might indicate a different and unreported antimicrobial compound could be produced.

The discovery of isolates with unique inhibition activity, unlike known antimicrobial compounds such as viridin, corynecin, weissellicin, and enterocin, suggests that there are still many undiscovered and unstudied antimicrobial compounds. This opens up the possibility for researchers to uncover new antimicrobial substances from human gut commensal bacteria and develop novel therapeutic approaches for infections by utilizing these bacteria as probiotics.

## Competing Interest

The authors declare that there is no conflict of interest.

## Author Contributions

AL and FG conceived the idea. AL, NK, JS, NHA, ENP, EZ, MS, and NDK designed the experiments. AL, AVA, and THM performed all the experiments. All authors contributed to the article and approved the submitted version.

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