

Antibacterial Activity of Cold Atmospheric Pressure Argon Plasma against 78 Genetically Different (*mecA*, *luk-P*, *agr* or Capsular Polysaccharide Type) *Staphylococcus aureus* Strains

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Key Words

Cold atmospheric pressure plasma · *Staphylococcus aureus* · Methicillin-resistant *S. aureus* · Virulence · Antibacterial efficacy · Tolerability

Abstract

Previous studies on the antimicrobial activity of cold atmospheric pressure argon plasma showed varying effects against *mecA*⁺ or *mecA*⁻ *Staphylococcus aureus* strains. This observation may have important clinical and epidemiological implications. Here, the antibacterial activity of argon plasma was investigated against 78 genetically different *S. aureus* strains, stratified by *mecA*, *luk-P*, *agr1–4*, or the cell wall capsule polysaccharide types 5 and 8. kINPen09[®] served as the plasma source for all experiments. On agar plates, *mecA*⁺ *luk-P*⁻ *S. aureus* strains showed a decreased susceptibility against plasma compared to other *S. aureus* strains. This study underlines the high complexity of microbial defence against antimicrobial treatment and confirms a previously reported strain-dependent susceptibility of *S. aureus* to plasma treatment.

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Introduction

Cold atmospheric pressure plasma (CAP), an argon plasma at ambient temperatures and in tissue-tolerable doses, has become an interesting treatment option for different infectious as well as non-infectious diseases [1, 2]. CAP has been investigated in the treatment of chronic wounds [3, 4], including chronic ulcers without [5] and in combination with the antiseptic octenidine [6] (which increased the efficacy of CAP due to its residual antimicrobial effect [7]), on skin graft donor wounds [8], for skin antisepsis [9–12], and for the inactivation of microorganisms embedded in biofilms [13–17].

The biological effects of kINPen09[®] (neoplas GmbH, Greifswald, Germany), one of the best-described plasma sources for the application of CAP to surfaces, are mainly caused by reactive oxygen and nitrogen species in the plasma flow or by plasma contact with liquids or organic substances [2, 9, 18, 19].

The antimicrobial activity of the kINPen09[®] has been investigated against a wide spectrum of microorganisms [20–24], and has demonstrated the ability to reduce the

Table 1. Overview on all investigated clinical *S. aureus* isolates (nasal carriage, blood culture, furunculosis, wound, lesion, pharyngeal or unknown origin) used in this study

Origin	Deduced MLST-CC	Name	<i>mecA</i>	<i>luk-P</i>	<i>agr</i> -type	<i>spa</i> -type ID	CP	Tested on agar	Tested on biofilm
Nasal carriage	CC5	SH 042-1	-	-	2	t002	5	•	
	CC5	SH 093-1	-	-	2	t002	5	•	
	CC5	sh08277	+	-	2	t010	5	•	
	CC5	sh02636	+	-	2	t151	5	•	
	CC7	SH 081-2	-	-	1	t091	8	•	
	CC8	SH 010-1	-	-	1	t008	5	•	
	CC8	T 169-1	-	-	1	t711	5	•	
	CC12	SH 059-1	-	-	2	t156	8	•	
	CC12	T 194-1	-	-	2	t156	8	•	
	CC15	SH 027-1	-	-	2	t084	8	•	
	CC15	SH 070-1	-	-	2	t084	8	•	
	CC22	330756	+	-	1	t608	5	•	
	CC22	SH 072-1	-	-	1	t005	5	•	
	CC22	SH 073-1	-	-	1	t005	5	•	
	CC22	sh12648	+	-	1	t020	5	•	
	CC22	sh13413	+	-	1	t032	5	•	
	CC22	sh18700	+	-	1	t032	5	•	
	CC22	sh19108	+	-	1	t020	5	•	
	CC22	sh19149	+	-	1	t032	5	•	
	CC22	sh05385	+	-	1	t608	5	•	
	CC22	SZ 255	-	-	1	t005	5	•	•
	CC22	SZ 296	-	-	1	t2816	5	•	
	CC25	SH 015-1	-	-	1	t056	5	•	
	CC25	T 192-1	-	-	1	t078	5	•	
	CC30	SH 039-1	-	-	3	t012	8	•	
	CC30	SH 079-1	-	-	3	t122	8	•	
	CC30	sh26625	-	-	3	t122	8	•	
	CC45	SH 001-2	-	-	1	t302	8	•	
	CC45	SH 013-2	-	-	1	t302	8	•	
	CC45	sh16309	+	-	1	t004	8	•	
	CC45	SZ 148	+	-	1	t1078	8	•	•
	CC59	SZ 179	+	+	1	t437	8	•	
	CC121	SZ 034	-	-	4	t435	8	•	
	CC121	SZ 275	-	-	4	t4495	8	•	•
CC395	T 110-1	-	-	1	t1651	8	•		
CC395	T 191-1	-	-	1	t1645	8	•		
Blood culture	CC7	BK 091	-	-	1	t091	8	•	
	CC7	BK 099	-	-	1	t091	8	•	
	CC8	98-00406	+	-	1	t051	5	•	•
	CC8	BK 010	-	-	1	t008	5	•	
	CC8	BK 077	-	-	1	t190	5	•	•
	CC12	BK 050	-	-	2	t160	8	•	
	CC12	BK 058	-	-	2	t156	8	•	
	CC15	BK 017	-	-	2	t084	8	•	
	CC15	BK 040	-	-	2	t094	8	•	
	CC22	BK 005	-	-	1	t1120	5	•	
	CC22	BK 090	-	-	1	t1665	5	•	
	CC25	BK 015	-	-	1	t056	5	•	
	CC25	BK 075	-	-	1	t078	5	•	
	CC30	BK 002	-	-	3	t012	8	•	
	CC30	BK 080	-	-	3	t021	8	•	•
	CC45	BK 020	-	-	1	t015	8	•	
	CC45	BK 051	-	-	1	t230	8	•	

Table 1 (continued)

Origin	Deduced MLST-CC	Name	<i>mecA</i>	<i>luk-P</i>	<i>agr</i> -type	<i>spa</i> -type ID	CP	Tested on agar	Tested on biofilm
	CC121	BK 036	-	-	4	t272	8	•	
	CC121	BK 078	-	-	1	t916	8	•	
	CC395	BK 066	-	-	1	t1362	8	•	
	CC395	BK 084	-	-	1	t1645	8	•	
Furuncle	CC1	H 3672	-	-	3	t177	8	•	
	CC1	H 5613	-	-	3	t127	8	•	
	CC5	H 7176	-	+	2	t002	5	•	
	CC8	05-01825	+	+	1	t008	5	•	•
	CC8	H 0757	-	+	1	t068	5	•	
	CC8	H 3163	-	+	1	t068	5	•	
	CC15	H 6110	-	-	2	t084	8	•	
	CC22	H 6754	-	+	1	t005	5	•	•
	CC22	H 9418	-	+	1	t1132	5	•	
	CC30	H 0678	-	+	3	t037	8	•	•
	CC30	H 2966	-	+	3	t318	8	•	
	CC45	H 3442	-	-	1	t116	8	•	•
	CC45	H 4764	-	-	1	t015	8	•	
	CC59	H 5391	+	+	1	t437	8	•	
	CC121	H 0070	-	+	4	t284	8	•	•
	CC121	H 4053	-	+	4	t435	8	•	
	CC121	H 5313	-	+	4	t159	8	•	
	CC121	H 5751	-	+	4	t272	8	•	
Wound	CC5	ATCC 29213	-	-	2	t002	5	•	
Pharyngeal	CC45	SZ 16315	+	-	1	t004	8	•	
Lesion	ST464	ATCC 6538	-	-	1	t3297	-	•	•

MLST-CC = Multi-locus sequence typing – clonal complex; CP = capsule polysaccharide.

number of microorganisms, such as methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* strains (MRSA, carrying the *mecA* gene) [25–28]. However, testing the efficacy of CAP on culture plates or biofilm showed varying effects, and details of the results pertaining to the application of plasma against *S. aureus* strains with or without the presence of the *mecA* gene indicate a possible increased tolerability of *mecA*⁺ strains [25, 27, 29]. This observation may have important clinical and epidemiological implications, since the application of CAP on chronic, multi-strain colonized wounds may result in the selection of antibiotic-resistant strains or bacterial strains with particular virulence factors. Furthermore, if this effect were dose dependent, the plasma dosage would need to be adjusted to achieve the necessary antimicrobial effect without selecting specific strains.

Based on the above, the aim of the present study was to investigate the antibacterial activity of CAP on 78 genetically different *S. aureus* strains, including MSSA and MRSA with and without Panton-Valentine leucocidin (*luk-P*), different *agr* types, and capsule polysaccharide types on culture plates or in biofilm.

Methods

Test Organisms

A total of 78 genetically different *S. aureus* strains were investigated in this study. Thereof, 19 *S. aureus* strains were obtained from the nasal cavity of asymptomatic carriers from Northern Germany (T, SH strains) [30], 6 strains were obtained from the nasal cavity of asymptomatic carriers from Szczecin, Poland (SZ strains) [31], 20 strains were obtained from patients from the University Hospital of Greifswald (BK strains) [30], and 17 strains were obtained from patients with furunculosis from a mature fu-

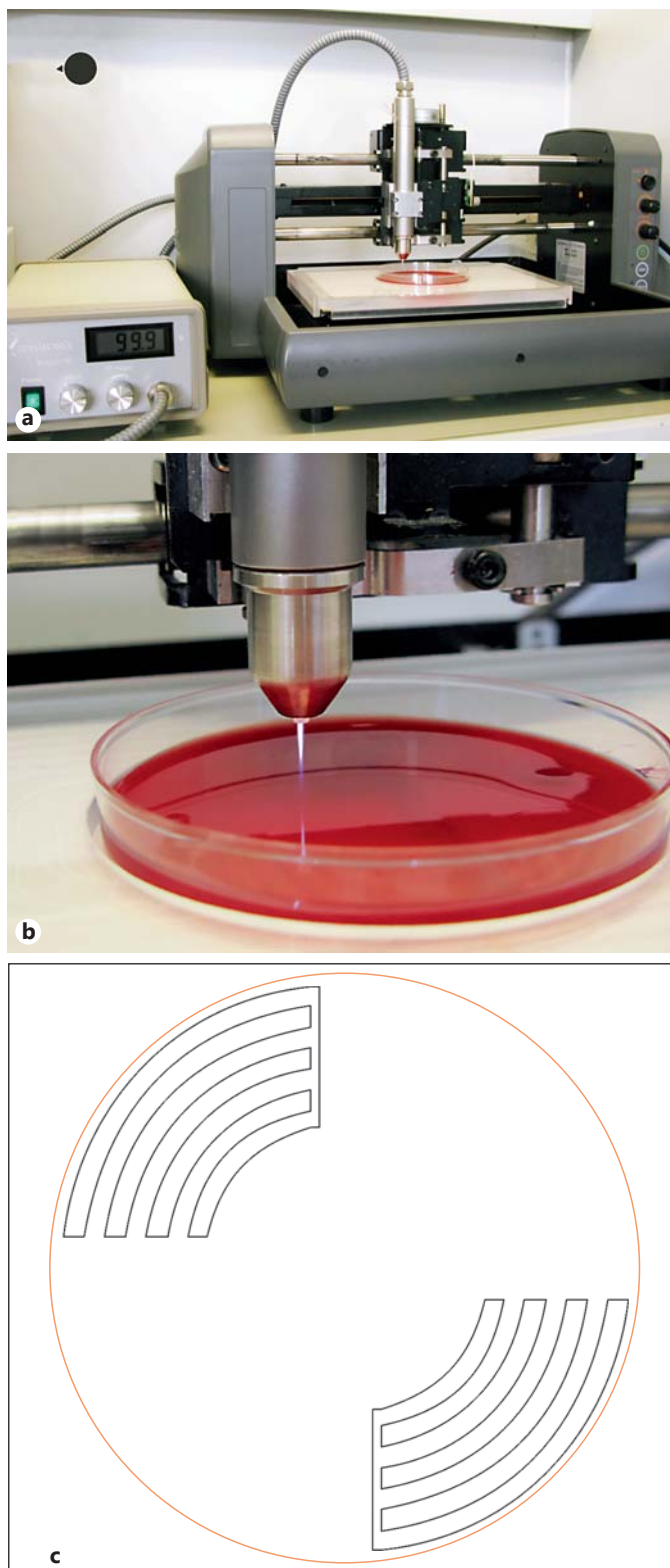


Fig. 1. Experimental set-up for the plasma application. The plasma pen is fixed above the blood agar plate (a) with, respectively, 10^2 or 10^4 CFU of *S. aureus* in a computer-controlled plotter (b). c Movement pattern for the plasma jet over the agar plate surface.

runcle during the acute phase of skin infection or by a surgeon during abscess incision, as described in Masiuk et al. [31] (H strains). All these strains were analysed for their clonal relationship (*spa* typing), and genotyped (multiplex PCR) for a range of virulence factors, methicillin resistance (*mecA*) and *luk-PV* genes [30, 31]. Based on this detailed characterization, we randomly selected strains from each clinical cohort (nasal colonization, blood culture, furunculosis) for subsequent analyses, representing the whole spectrum of virulence factors (table 1).

Moreover, we included 2 ATCC (American Type Culture Collection) reference MSSA strains (ATCC 6538 and ATCC 29213), as well as 12 MRSA isolates of healthy carriers, obtained from the currently ongoing Study of Health in Pomerania (SHIP study), a large-scale, comprehensive population-based study in Western Pomerania (table 1) [32]. Out of these 12 MRSA strains, 1 isolate originated from a throat swab (sh16315), while the other 11 isolates were obtained from the nasal cavity.

Two isolates, strain 05-01825 (*mecA*⁺, *luk-P*⁺) and strain 98-00406 (*mecA*⁺, *luk-P*⁻), were obtained from the Robert Koch Institute, Wernigerode, Germany (table 1) and were analysed by Strommenger et al. [33]. Screening of the susceptibility of all *S. aureus* strains was carried out on Columbia blood agar plates (BD, Heidelberg, Germany). Based on different *spa* clonal complex clusters (CC8, CC22, CC30, CC45, CC121) and the prevalence of *mecA* and *luk-P* within these clusters, 12 selected strains were used for experiments on biofilms (table 1). The capsular polysaccharides were deduced in accordance with the multi-locus sequence typing cluster of each strain [34–37].

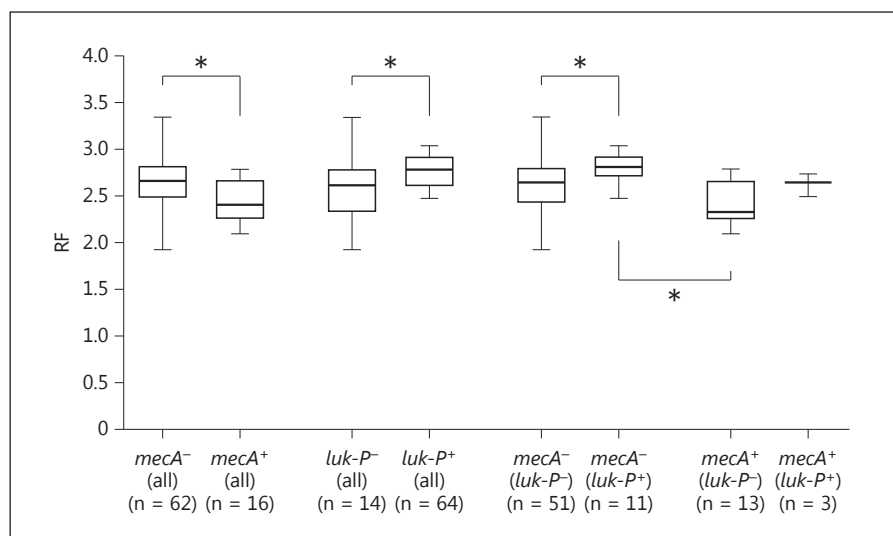
Bacterial Cultivation and Preparation for Plasma Application

The test organisms used for stock- and subculture were stored at -70°C attached onto glass beads. For cultivation, the test organisms were maintained on Columbia blood agar (BD) for 24 h at 37°C . The stock cultures were stored at 4°C for maximal 4 weeks to prepare the subcultures. The subcultures were prepared on the day before the experiment. A loopful of grown bacteria was diluted in 5 ml of 0.9% (v/w) PBS solution (PAA Laboratories, Cölbe, Germany). This suspension corresponded to an initial concentration of 10^8 colony-forming units (CFU)/ml. After dispersion by whirling for 1 min (Vortex-Genie 2; Scientific Industries, New York, N.Y., USA), the suspension was serially diluted in sterile PBS. For the experiments, 10^4 CFU/ml for plasma application and 10^2 CFU/ml for the treatment with argon gas (gas control) and the control (untreated test organisms) were used. The used dilutions were determined in pre-tests to obtain countable quantities of CFU after treatment. For standardized spreading of the bacteria on agar plates 50 μl of the corresponding suspension were applied by a spiral plater (Meintrup DWS, Lähden-Holte, Germany). The inoculated plates were incubated for 20 min at room temperature to get homogeneously dry surfaces.

Plasma Treatment

A cold atmospheric pressure argon plasma pen (kINPen09[®]; neoplas GmbH), using argon (99.995% pure) as carrier gas with a gas flow rate of 5 slm (standard litres/min), was used as the plasma source to generate spatial afterglow plasma. The gas flow was controlled by a mass flow controller (MKS Instruments, Munich, Germany). The plasma pen frequency was set at 1.1 MHz with a maximum input DC power of 3.5 W to the hand-held unit [38]. At this input power a mean heat output of about 300 mW is generated on

Fig. 2. Impact of *mecA* and *luk-P* on the reduction of CFU/ml in \log_{10} of 78 different *S. aureus* strains on sheep blood agar after plasma treatment. The *mecA*^{+/-} and *luk-P*^{+/-} subgroups were compared. Box-plot graph depict the median, 25th percentile, minimum and maximum, and number of values (respective number of strains) of each group. Assays were performed in duplicate. The asterisks indicate statistically significant difference between the respective groups.



the surface [38]. The generated plasma jet was directed at the treated surface open to the indoor air during treatment.

For all experiments, the plasma pen was fixed in a computer-controlled x/y/z table (modified EDX-20; Roland DG, Westerlo, Belgium; fig. 1a). Each prepared agar plate was positioned at a distance of 7 mm below the nozzle of the pen (fig. 1b). Two quarters of the plate were treated in a curved meander at a speed of 10 mm/s 10 times, resulting in a total treatment time of approximately 6 s per point. The distance between the meandering lines was 3 mm (fig. 1c). The temperature on the surface during treatment did not exceed 41°C. Each strain and treatment type, including the gas (without plasma) and untreated controls, was performed twice. After treatment, the agar plates were incubated for 24 h at 37°C. Treatment of the polystyrene-grown biofilms of the selected 12 strains was carried out at least 8 times for the control, 10 times for plasma treatment and 3 times the gas control (gas flow without plasma), as described previously by Matthes et al. [39]. From the 78 strains screened, a sample of 12 strains was selected for biofilm tests to represent different clonal complexes, capsular polysaccharide types (5 and 8), and variable presence of *mecA* and *luk-P*.

Statistical Analyses

CFU were calculated using the Spiral[®] counting grid according to the manufacturer's instructions (Meintrup DWS) and were expressed in \log_{10} (CFU/ml). CFU of the treated plates were compared with the median of the non-treated control plates of each test. The \log_{10} reduction factor (RF \log_{10}) was defined by the formula:

$$\text{RF } \log_{10} = \log_{10}(mc) - \log_{10}(vs)$$

where 'mc' described the median of the (untreated) control values of each test organism and 'vs' the single value of the treated sample (plasma or gas alone). All tests on Columbia agar plates were conducted in duplicate, and all tests on biofilm were repeated 10-fold. The standard deviations and p values ($\alpha = 0.05$) were calculated for the RFs. Statistical differences were analysed using the Kruskal-

Wallis test, followed by Dunn's multiple comparison test or the Mann-Whitney U test to analyse two groups with each other, such as *mecA*⁺ versus *mecA*⁻ and variants of *luk-P*⁻ versus *luk-P*⁺, or capsular polysaccharide 5 versus capsular polysaccharide 8 (Prism; GraphPad, La Jolla, Calif., USA).

Results

The average reduction of CFU for *S. aureus* strains on Columbia blood agar after plasma treatment was 2.59 ± 0.25 RF \log_{10} . Gas treatment (without plasma) achieved a non-significant reduction of RF \log_{10} 0.28 ± 0.16 and was significantly less effective compared to plasma treatment. The *mecA*⁺ (all) *S. aureus* strains were significantly less susceptible to CAP than the *mecA*⁻ (all) strains, with a difference of 0.2 \log_{10} ($p < 0.01$). Additionally, strains of *mecA*⁺ (all), *mecA*⁺ *luk-P*⁻, and *mecA*⁻ *luk-P*⁻ strains showed a statistically significantly lower susceptibility against CAP compared to *mecA*⁻ *luk-P*⁺ strains, with a difference of 0.4 or 0.2 CFU/ml ($p < 0.05$; fig. 2).

After a comparison of the *S. aureus* strains on the basis of the *agr* type, the *agr3* group was statistically more susceptible to CAP than the *agr1* and *agr2* groups ($p < 0.05$; fig. 3). In contrast, grouping the isolates by the capsular polysaccharide types 5 and 8, which are linked to *S. aureus* lineages, did not show any statistically significant difference after plasma treatment on agar plates (fig. 3). No significant difference was shown ($p > 0.05$) between the clonal complex clusters (CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45, CC121, CC395).

Table 2. Average RF (RF log₁₀) of tested *S. aureus* strains after argon plasma treatment on Columbia agar plates (n = 78) and biofilms (n = 12)

Genetic background	Test organisms after plasma treatment			
	agar plates		biofilms	
	n	RF±SD	n	RF±SD
<i>mecA</i> ⁻ (all)	62	2.67±0.27 ^a	9	1.77±0.24
<i>mecA</i> ⁺ (all)	16	2.41±0.23 ^b	3	1.99±0.20
<i>luk-P</i> ⁻ (all)	64	2.62±0.28 ^c	8	1.83±0.20
<i>luk-P</i> ⁺ (all)	14	2.78±0.17	4	1.87±0.29
<i>mecA</i> ⁻ (<i>luk-P</i> ⁻)	51	2.65±0.28 ^b	6	1.83±0.20
<i>mecA</i> ⁻ (<i>luk-P</i> ⁺)	11	2.81±0.17	3	1.75±0.35
<i>mecA</i> ⁺ (<i>luk-P</i> ⁻)	13	2.33±0.24 ^b	2	1.90±0.27
<i>mecA</i> ⁺ (<i>luk-P</i> ⁺)	3	2.65±0.12	1	1.99
<i>agr1</i>	47	2.64±0.27	8	1.94±0.24
<i>agr2</i>	15	2.57±0.23		
<i>agr3</i>	9	2.83±0.23 ^d	2	1.63±0.00
<i>agr4</i>	7	2.60±0.25	2	1.85±0.15
CP 5	33	2.60±0.28	5	1.99±0.27
CP 8	44	2.67±0.25	6	1.73±0.18

SD = Standard deviation; CP = capsule polysaccharide.

^a Significantly different from *mecA*⁺ (all) on agar plates. ^b Significantly different from *mecA*⁻ (*luk-P*⁺) on agar plates. ^c Significantly different from *luk-P*⁺ (all) on agar plates. ^d Significantly different from the *agr1*, *agr2* group, and all *luk-P*⁻ groups on agar plates.

Plasma treatment of the 12 selected *S. aureus* strains embedded in biofilm showed an average reduction of RF log₁₀ 1.86 ± 0.22. Gas treatment alone resulted in an average RF log₁₀ of 0.77 ± 0.25 and was not significantly different from the respective controls (p > 0.05). Here, the average median of the biofilm controls was 8.36 ± 0.23 log₁₀ CFU/ml. Within this strain cohort, we did not observe a difference in susceptibility to gas treatment between *mecA*⁻ or *mecA*⁺ strains, *luk-P*⁺ and *luk-P*⁻ strains, the *agr* group, or the group of the capsular polysaccharide types 5 and 8 (table 2). No significant difference was shown (p > 0.05) between the clonal complex clusters (CC8, CC30, CC45, CC88, CC121). Detailed results for both test conditions are summarized in table 2. The RF values of the agar- and biofilm-grown *S. aureus* did not correlate, and no segregation of the *mecA*, *luk-P*, *agr*, and capsular polysaccharide subgroups was observed (data not shown).

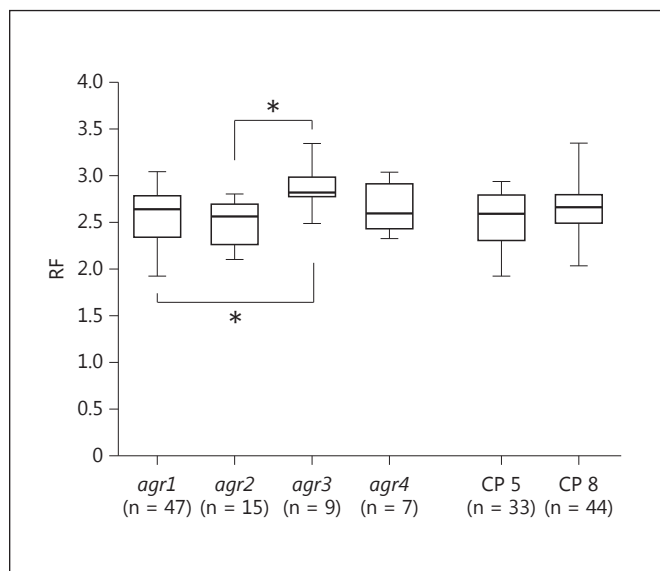


Fig. 3. Impact of *agr* and type of capsular polysaccharide (CP) on the reduction of CFU/ml in log₁₀ of 78 different *S. aureus* strains on sheep blood agar after plasma treatment. The *agr1*–4 and capsular polysaccharide subgroups were compared. Boxplot graph depicting the median, 25% percentile, minimum and maximum, and number of values (respective number of strains) of each group. Assays were performed in duplicate. The asterisks indicate statistically significant difference between the respective groups.

Discussion

Different researchers have investigated the effect of ‘cold’ atmospheric pressure plasma on drug-resistant bacteria such as MRSA in planktonic conditions, adhered on surfaces, or embedded in biofilm [25, 26, 28, 29, 40, 41]. Some of these studies showed differences in the susceptibility of MRSA and MSSA strains to plasma treatment for the planktonic form and after biofilm cultivation, with an increased survival rate of approximately 6% after treatment for 2 min [27], and from 5 up to 70% after treatment for 3 min on agar plates [24, 25]. Other authors reported an increased susceptibility of 1 MRSA and 1 MSSA strain compared to 2 MRSA strains (USA300 and USA400) in planktonic conditions [29]. However, embedded in biofilm, opposite results were observed. The different analysis methods used in the study conducted by Joshi et al. [29] complicate a direct comparison between agar- and biofilm-treated strains, yet indicate that the different results may be caused by variations in the bacterial environment and the analysis method used.

Our study confirms previously reported strain-dependent variances in the susceptibility of *S. aureus* against CAP. Consistent with observations by the other authors, *mecA*⁺ *luk-P*⁻ *S. aureus* strains showed a decreased susceptibility against CAP when grown on agar plates. In biofilms, however, no significant difference could be determined for all investigated factors. This may be due to the limited cohort size used in our study or may reflect the effect of the biofilm form, which outnumbers the effect of variances between strains.

One reported explanation for the observations after *S. aureus* treatment on agar plates could be the differences in the general stress response and membrane lipid and/or protein composition [27]. It is also known that the cell wall structure of microorganisms influences the diffusion of reactive oxygen species and therefore the antimicrobial efficacy of plasma [42]. Actually, our results demonstrate no statistical difference between the genetic background in producing capsule polysaccharide type 5 or 8 for *S. aureus* on agar plates or in biofilm. This suggests that the capsular polysaccharide type does not have an influence on the diffusion of plasma compounds through the cell wall and on the antimicrobial reactivity of CAP, and underpins that differences shown by various microbial species could be mainly caused by the cell wall thickness [42].

Besides the oxidation processes of polysaccharides and lipids of the bacterial cell wall and membrane by reactive oxygen plasma compounds [28, 42–44], plasma can induce a general stress response to the reactive products of the air-liquid-substrate interaction of plasma [45]. Hence, the stress response towards plasma could be influenced by the different genetic backgrounds, resulting in different metabolic activities or differences in cell wall structure depending on growth on agar or in a biofilm.

However, it is also known that the acquisition of mobile genetic elements encoding additional resistances or virulence factors could decrease the proliferation rate, fitness, and survival in the environment [46–48]. In our study, 3 different pathogenic markers were used to compare their influence on the antibacterial effect of CAP. The phage-mediated virulence gene *luk-PV* can express the pore-forming toxin Panton-Valentine leucocidin, which is associated with furunculosis and fulminant staphylococcal pneumonia [49]. The expression of the chromosomal cassette-encoded *mecA* leads to methicillin-resistant pathogens [50]. The global regulator gene (*agr*) regulates the expression of different virulence genes; it is involved in the expression of different capsular polysaccharides, capsular proteins and biofilm development

[50–53], and can influence the pathogenesis of infections [54].

Reduced susceptibility against CAP of *mecA*⁺ strains was enhanced by the absence of *luk-P* (*luk-P*⁻). The same influence of *luk-P* was also observed by the MSSA strains. Here, the ability for the expression of *mecA* seems to protect against the influence of CAP, whereas *luk-P* or *agr3* increase the sensitization against CAP. These results show that virulence and resistance genes can influence tolerability against plasma treatment. Even though the statistically significant differences of determined CFU show the complexity of the cell responses by CAP, they are microbiologically negligibly low and would not be relevant in clinical use.

Our study is limited by a number of factors. First, a selection bias may have occurred as we used clinical isolates which had been collected during previous, ethics committee-approved studies. Second, although we tried to select subsets of strains for subanalysis based on their genetic/virulence profile, a matched-pair analysis was not possible because of the heterogeneity of the test material. This eventually also led to low numbers of isolates (e.g. in the *mecA*⁺ *luk-P*⁺ group).

Our results indicate the influence of the genetic background on the antimicrobial effectiveness of plasma, which may be influenced by the variable expression of these genes in the different clinical isolates. The gene expression can vary dramatically under in vitro and in vivo conditions [47, 55].

Conclusion

In conclusion, our results underline the high complexity of microbial defence against antimicrobial treatment. Differences in the achieved reductions after the application of CAP were observed. The clinical relevance of these observations remains unclear and may warrant further research, since the role of distinct genetic characteristics may have an impact on the selection and virulence of microorganisms under habitat conditions.

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Disclosure Statement

The authors declare no conflicts of interest.

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