

# Efficacy of taurolidine against periodontopathic species—an in vitro study

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**Abstract** The antimicrobial effect of taurolidine was tested against periodontopathic species in comparison to chlorhexidine digluconate in the presence or absence of serum. Minimal inhibitory concentrations (MIC), microbiocidal concentrations (MBC), as well as killing were determined against 32 different microbial strains including 3 *Porphyromonas gingivalis*, 3 *Aggregatibacter actinomycetemcomitans*, and 15 potentially superinfecting species with and without 25% v/v human serum. The MIC<sub>50</sub> of taurolidine against the tested microbial strains was 0.025% and the MIC<sub>90</sub> 0.05%. The respective values for the MBCs were 0.05% and 0.1%. Addition of 25% serum (heat-inactivated) did not change the MIC and MBC values of taurolidine. In contrast, MICs and MBCs of chlorhexidine (CHX) increased by two steps after addition of serum. Taurolidine killed microorganisms in a concentration and time-dependent manner, the killing rate of 1.6% taurolidine was 99.08%±2.27% in mean after 2 h. Again, killing activity of taurolidine was not affected if serum was added, whereas addition of inactivated serum clearly reduced the killing rate of all selected bacterial strains by CHX. Therefore, taurolidine possesses antimicrobial properties which are not reduced in the presence of serum as a main

component in gingival crevicular fluid and wound fluid. Taurolidine may have potential as an antimicrobial agent in non-surgical and surgical periodontal treatment.

**Keywords** Taurolidine · Chlorhexidine · Periodontopathic bacteria · Serum

Taurolidine is a derivative of the amino acid taurine. Taurine is not incorporated into proteins. It plays a role in the development of the central nervous system, the retina membrane stabilization, and immune response. The level of the enzyme cysteine sulfinic acid decarboxylase which is required for biosynthesis of taurine is low in humans. Therefore, the intake of taurine occurs via food, i.e., it is found especially in seafood and meat [1]. Decreased plasma levels of taurine have been reported in trauma and sepsis [2]. Taurolidine acts as an antibacterial agent and prevents adhesion of bacteria to epithelial cells [3]. Taurolidine inhibits production of proinflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-α [4]. Animal studies with cats fed a taurine-free diet resulted in a significant reduction of phagocytosis rate of *Staphylococcus epidermidis* [5]. Hamsters treated with supplementary taurine did not develop typical oxidant tissue damage after NO<sub>2</sub> exposure [6]. Taurine reacts with HOCl and produces the less reactive and more stable taurine chloramines [1]. The activity of taurolidine is not influenced by organic matter such as blood [7]. The systemic toxicity of taurolidine appears to be low indicated by the finding that healthy volunteers were administered 5 g of taurolidine per day without serious adverse effects [8]. In vitro, toxic effects were observed after 30 min exposure of 0.5% taurolidine to chondrocytes but still being less in comparison to 3% hydrogen peroxide and 0.04% polyhexanide [9].

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Taurolidine is used in the prevention of catheter-related infections [10] and in bone infections [11]. A possible application is discussed in the treatment of tumors since it has been shown that taurolidine promotes apoptosis of tumor cells [12].

Due to its properties, taurolidine may be also a promising disinfectant in the therapy of periodontitis and peri-implantitis. However, at present, chlorhexidine (CHX) represents the gold standard among the disinfectants used for the treatment of periodontal and peri-implant infections. Chlorhexidine is active against many microbial species [13–15], but it may exert some cytotoxic effect as shown in some *in vitro* studies [16, 17]. Furthermore, the antimicrobial activity of chlorhexidine is strongly reduced in the presence of serum [18], which in turn, limits its effectiveness in the subgingival environment. Therefore, there is a stringent need to search for novel agents exhibiting an antimicrobial activity in the presence of serum or blood thus having a potential for clinical application in the subgingival environment. Although taurolidine may represent a potential agent against periodontopathic bacteria, the available data are still very limited. Therefore, the aim of this *in vitro* study was to evaluate the antimicrobial effect of taurolidine against selected periodontopathic species in comparison to chlorhexidine digluconate in the presence or absence of serum.

## Materials and methods

### Antimicrobials

Taurolidine was available as a 2% solution, which, according to the manufacturer's information, was the highest concentration to be solubilized (Geistlich Pharma AG, Wolhusen, Switzerland), while 0.1% chlorhexidine digluconate (CHX) solution (B. Braun, Melsungen, Germany) was used as a positive control. Different dilutions with dH<sub>2</sub>O were made from these concentrations. Sodium chloride (0.9%) served as the negative control.

### Microorganisms

In total, 32 different microbial strains were included in the study. Seventeen of these strains belonged to species clearly involved in pathogenesis of periodontitis (“real” periodontopathogens); the 15 others may play a role as potentially superinfecting species. Among them, 11 bacterial strains were Gram-positive, 20 Gram-negative, and 1 strain was a yeast. Beside laboratory strains, clinical isolates were included. Three *P. gingivalis* strains, three *A. actinomycetemcomitans* strains, as well as six potentially superinfecting species, originated from clinical samples obtained from patients with severe periodontitis (Table 1).

All the strains were precultivated 24–72 h prior to the experiments. Modified tryptic soy agar [19] (“real” periodontopathic bacteria) and tryptic soy agar were used as cultivation media. *A. actinomycetemcomitans* strains and *Streptococcus constellatus* ATCC 27823 were always incubated with 5% CO<sub>2</sub>, the other “real” periodontopathogens were incubated anaerobically and all potentially superinfecting species within normal atmospheric conditions, each at 37°C.

### Minimal inhibitory and minimal microbiocidal concentrations

Micro-broth dilution technique was used to determine minimal inhibitory concentrations (MICs). After subcultivation of bacterial strains (and a yeast species) and checking of purity, a defined inoculum was added to a broth containing defined concentrations of the antimicrobials. The range of the tested final concentrations were 0.0004–0.025% for chlorhexidine digluconate and 0.00625–0.4% for taurolidine. Mueller-Hinton broth was used for the superinfecting species and Wilkins-Chalgren broth added by 2.5 mg l<sup>-1</sup> vitamin K, 5 mg l<sup>-1</sup> hemin, 10 mg l<sup>-1</sup> N-acetylmuramic-acid, and 5% blood was the medium used for the others. After an incubation time of 42 h (18 h aerobes), the growth of microbes was analyzed by visual checking of turbidity. The MIC represented the lowest concentration without visible turbidity. MIC<sub>50</sub> and MIC<sub>90</sub> data are the values for MICs where 50% and 90% of the strains were inhibited in their growth. In case of Wilkins-Chalgren broth with all the additives, it was difficult to register visually turbidity. Here, subcultivation of all wells was made, and MIC was defined as the concentration showing clearly less growth than the control. For determination of minimal microbiocidal (mostly bactericidal) concentration (MBC), non-turbid cultures were subcultivated on agar plates without the addition of any antimicrobial agent. After incubation, MBCs were measured as the lowest concentration without any colonies on agar plates. MBC<sub>50</sub> and MBC<sub>90</sub> represent the concentrations where 50% and 90% of the strains were completely killed.

Determination of MICs and MBCs were repeated in the presence of 25% *v/v* inactivated human serum (Sigma-Aldrich, Steinheim, Germany). Serum had been inactivated by heating to 56°C for 30 min to determine exclusively the inhibitory effect of serum proteins and to exclude a killing by the complement cascade as a component of the serum. Native serum (without inactivation) was additionally used for six selected species to consider the possible bactericidal effect of complement in addition to the serum proteins. The following bacterial strains were included in these experiments: *A. actinomycetemcomitans* J7, *Fusobacterium*

**Table 1** Tested microorganisms and their characteristics (origin, Gram characteristics)

Species	Origin	Gram
“Real” periodontopathic		
<i>Fusobacterium nucleatum</i> ATCC 25586	Laboratory strain	Negative
<i>Prevotella intermedia</i> ATCC 25611	Laboratory strain	Negative
<i>Porphyromonas gingivalis</i> ATCC 33277	Laboratory strain	Negative
<i>P. gingivalis</i> M5-1-2	Clinical isolate	Negative
<i>P. gingivalis</i> MaRL	Clinical isolate	Negative
<i>P. gingivalis</i> J430-1	Clinical isolate	Negative
<i>Tannerella forsythia</i> ATCC 43037	Laboratory strain	Negative
<i>Aggregatibacter actinomycetemcomitans</i> ATCC 33384	Laboratory strain	Negative
<i>A. actinomycetemcomitans</i> J1	Clinical isolate	Negative
<i>A. actinomycetemcomitans</i> J2	Clinical isolate	Negative
<i>A. actinomycetemcomitans</i> J7	Clinical isolate	Negative
<i>Campylobacter rectus</i> ATCC 33238	Laboratory strain	Negative
<i>Eikenella corrodens</i> ATCC 23834	Laboratory strain	Negative
<i>Capnocytophaga gingivalis</i> ATCC 33624	Laboratory strain	Negative
<i>Eubacterium nodatum</i> ATCC 33099	Laboratory strain	Positive
<i>Parvimonas micra</i> ATCC 33270	Laboratory strain	Positive
<i>Streptococcus constellatus</i> ATCC 27823	Laboratory strain	Positive
Potentially superinfecting species		
<i>Enterobacter cloacae</i> JGr1	Clinical isolate	Negative
<i>Klebsiella pneumonia</i> JGr2	Clinical isolate	Negative
<i>Pseudomonas aeruginosa</i> DSM 50071	Laboratory strain	Negative
<i>Escherichia coli</i> VA25304/2-09 (ESBL)	Clinical isolate	Negative
<i>E. coli</i> VA25488/1-09 (ESBL)	Clinical isolate	Negative
<i>E. coli</i> BK 20303-09 (ESBL)	Clinical isolate	Negative
<i>Enterococcus faecalis</i> ATCC 29212	Laboratory strain	Positive
<i>Staphylococcus aureus</i> ATCC 29213	Laboratory strain	Positive
<i>S. aureus</i> ATCC 43300 (MRSA)	Laboratory strain	Positive
<i>S. aureus</i> MR8126-09 (MRSA)	Laboratory strain	Positive
<i>S. aureus</i> VA25607/2-09 (MRSA)	Clinical isolate	Positive
<i>Enterococcus faecium</i> VA23477/1-09	Clinical isolate	Positive
<i>E. faecium</i> UR17400-09	Clinical isolate	Positive
<i>E. faecium</i> ST10343-09	Clinical isolate	Positive
<i>Candida albicans</i> ATCC 76615	Laboratory strain	Yeast

*nucleatum* ATCC 25586, *P. gingivalis* ATCC 33277, *S. constellatus* ATCC 27823, *Enterobacter cloacae* JGr1, and *Staphylococcus aureus* MRSA VA25607/2.

#### Killing activity

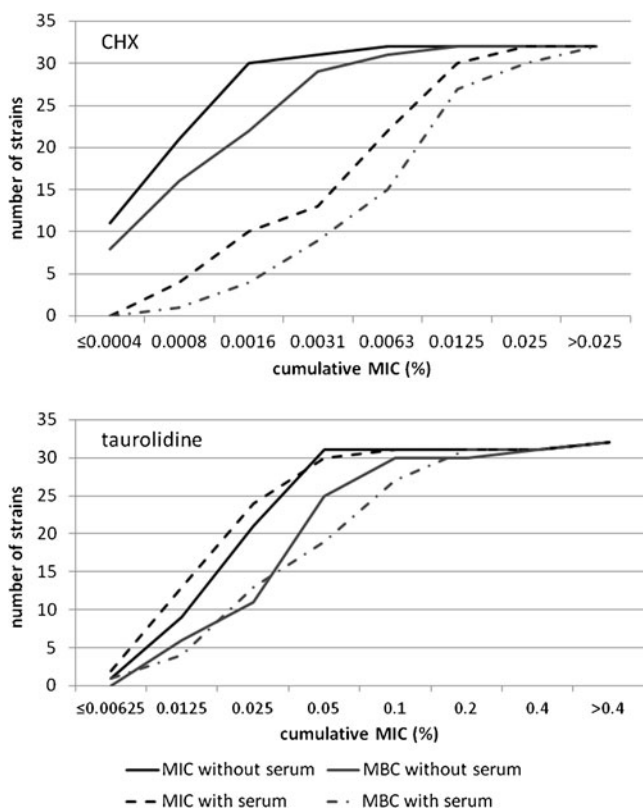
A defined inoculum of microorganisms (about  $10^4$ ) in 0.9% w/v NaCl with 0.01% (w/v) yeast extract was added by taurolidine and CHX in different concentrations (CHX—0.005%, 0.02%, 0.008%; taurolidine—0.1%, 0.4%, 1.6%). After 1 and 2 h of incubation, the numbers of viable bacteria were determined by enumeration of colony forming units. Determination of killing rates was repeated for six species in the presence of 25% v/v inactivated and native

serum. The same bacterial strains which were included in the MIC and MBC experiments with native serum were tested. Here, the lowest concentration of each antimicrobial was chosen, only *P. gingivalis* ATCC 33277 was tested in the presence of 0.4% taurolidine; without serum, 0.1% taurolidine did not show any killing effect.

All experiments were made in independent duplicate. Methods were adapted to the guidelines of Clinical and Laboratory Standards Institute [20, 21].

#### Scanning electron microscopy photographs

Four strains (*F. nucleatum* ATCC 25586, *P. gingivalis* ATCC 33277, *A. actinomycetemcomitans* J7, *S. aureus*



**Fig. 1** Cumulative minimal inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) of chlorhexidine digluconate and taurolidine against all tested strains ( $n=32$ ) with and without serum. MICs and MBCs were determined by using micro-broth dilution technique. Serum was added in a final concentration of 25% v/v after being inactivated to exclude a potential bactericidal effect of complement

VA25607/2-09) were chosen for scanning electron microscopy (SEM) photographs. An overnight culture was placed on slides and exposed to aqueous solutions containing 0.08% CHX and 1.6% taurolidine solutions and dH<sub>2</sub>O (control) for 1 h at 37°C. After removal of the solution, the bacteria were carefully washed with dH<sub>2</sub>O and initially

fixed in 2% glutaraldehyde in cacodylate buffer for 30 min, washed twice with cacodylate buffer and dehydrated using a graded ethanol series (10 min each concentration). Following critical point drying, samples were sputtercoated with gold and examined with a ZEISS LEO-1530 Gemini (Carl Zeiss NTS GmbH, Oberkochen, Germany) equipped with a field emission electron gun at 10 keV.

## Results

Determination of minimal inhibitory and minimal microbiocidal concentrations with and without serum

The MIC<sub>50</sub> of CHX against the tested microbial strains was 0.0008% and the MIC<sub>90</sub> 0.0016%. The respective values for MBC<sub>50</sub> and MBC<sub>90</sub> were 0.0008% and 0.0063%. Addition of 25% serum (heat-inactivated) enhanced the MIC and MBC values by up to three steps. The MIC<sub>50</sub> and MIC<sub>90</sub> with serum were 0.0063% and 0.0125%. The MBC<sub>50</sub> and MBC<sub>90</sub> values were 0.0125% and 0.025%. The MIC<sub>50</sub> of taurolidine against the tested microbial strains was 0.025% and the MIC<sub>90</sub> 0.05%. The respective values for the microbiocidal concentrations were 0.05% and 0.1%. Addition of 25% serum (heat-inactivated) did not enhance the MIC and MBC values in general. The MIC<sub>50</sub> and MIC<sub>90</sub>, as well as the MBC<sub>50</sub> with serum, were the same as without. Only the MBC<sub>90</sub> value was one step higher and reached 0.2% (Fig. 1).

Additionally, the effect of native (non-inactivated) serum was tested on six selected species. In mean, MIC of CHX increased by two steps after addition of non-activated serum. Using native serum resulted in the same MICs for four strains, unexpectedly, a higher MIC was registered for *S. constellatus* ATCC 27823 compared to inactivated serum. MBCs of CHX increased at least by two steps after addition of non-activated serum. Using native serum resulted in the same MBCs for three strains, lower MBCs

**Table 2** Minimal inhibitory concentrations of chlorhexidine digluconate against selected species without, as well as with inactive and native serum

Strain	MIC/MBC of chlorhexidine without serum	with inactivated serum	with native serum
<i>A. actinomycetemcomitans</i> J7	0.0016/0.0016	0.0063/0.0063	0.0063/0.0063
<i>F. nucleatus</i> ATCC 25586	≤0.0004/≤0.0004	0.0031/0.0031	0.0031/0.0031
<i>P. gingivalis</i> ATCC 33277	≤0.0004/≤0.0004	0.0008/0.0016	0.0016/0.0016
<i>S. constellatus</i> ATCC 27823	0.0004/0.0004	0.0016/0.0031	0.0250/0.0500
<i>E. cloacae</i> JGr1	0.0063/0.0063	0.0250/0.1000	0.0250/0.0250
<i>S. aureus</i> MRSA VA25607/2	0.0016/0.0031	0.0063/0.0125	0.0063/0.0063

Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined by using micro-broth dilution technique. Serum was added in a final concentration of 25% v/v native and after being inactivated to exclude a potential bactericidal effect of complement

**Table 3** Minimal inhibitory concentrations of taurolidine against selected species without, as well as with inactive and native serum

Strain	MIC/MBC of taurolidine without serum	MIC/MBC of taurolidine with inactivated serum	MIC/MBC of taurolidine with native serum
<i>A. actinomycetemcomitans</i> J7	0.0125/0.0125	0.0125/0.0125	≤0.0063/0.0125
<i>F. nucleatum</i> ATCC 25586	0.0125/0.0125	0.0125/0.0125	0.0125/0.0125
<i>P. gingivalis</i> ATCC 33277	0.0125/0.0250	0.0125/0.0250	0.0125/0.0125
<i>S. constellatus</i> ATCC 27823	≤0.0063/0.0063	≤0.0063/0.0063	0.0125/0.0125
<i>E. cloacae</i> JGr1	0.0500/0.1000	0.0500/0.1000	0.0500/0.0500
<i>S. aureus</i> MRSA VA25607/2	0.0500/0.0500	0.0250/0.1000	0.0250/0.0250

were registered for the two involved potentially superinfecting species and *S. constellatus* ATCC 27823 was less susceptible compared to inactivated serum (Table 2). The MICs of taurolidine did not increase after addition of inactivated serum, contrary *S. aureus* MRSA VA25607/2 was slightly more susceptible. Using native serum resulted in the same MICs for four strains, the MICs of two other strains changed only slightly by one step (one MIC was higher, the other lower). In general, MBCs of taurolidine did not change after addition of inactivated serum; only the MBC of *S. aureus* MRSA VA25607/2 was one step higher than without. Using native serum resulted in the same MBCs for two strains, lower MBCs were registered for the two involved potentially superinfecting species as well as for *P. gingivalis* ATCC 33277, and only *S. constellatus* ATCC 27823 was slightly less susceptible compared to the inactivated serum (Table 3).

### Killing

CHX was highly efficient in killing the tested microorganisms. In mean, the killing rate was more than 99% for all three tested concentrations after 2 h. For taurolidine, a concentration and time-dependent effect was visible. Nevertheless, the killing rate of the highest tested concentration of 1.6% was 99.08%±2.27% after 2 h (Fig. 2). All concentrations of CHX and the highest tested concentration of taurolidine (1.6%) completely eradicated most of the strains after 2 h. Here, taurolidine eliminated all Gram-negative potentially superinfecting strains (Table 4). Addition of inactivated serum clearly reduced the killing rate of all selected bacterial strains by CHX; moreover, *F. nucleatum* ATCC 25586 was obviously stimulated to multiply. Compared to the inactivated serum, native serum increased the killing rates of all species. Serum did not influence clearly the killing ability of taurolidine, except for *E. cloacae* JGR1, where it was reduced (Fig. 3).

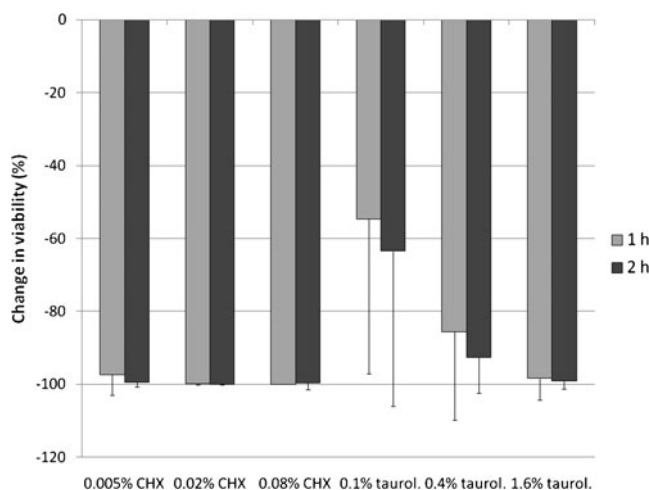
### SEM photographs

Three Gram-negative and one Gram-positive species were chosen for electron microscopy. Gram-negative bacteria

having an outer membrane seemed to be more damaged by the antimicrobials. After exposure to chlorhexidine, a flow out of substances is clearly visible. In part, bacteria seemed to have burst. The damages were not as obvious after exposure to taurolidine. The surface appeared to be rough; impressions suggest also a flow out of inner particles, but to a much less extent, compared to chlorhexidine. The surface of *S. aureus* VA25607-02/09 (MRSA) appeared unchanged after exposure of the antimicrobials. An outer membrane is missing in Gram-positives, and the inner membrane is covered by a thick peptidoglycan layer. Thus, possible damages to the inner membrane might not be visible (Fig. 4).

### Discussion

The antimicrobial activity of taurolidine has been known for more than 20 years. It was first used in the prevention and treatment of peritonitis [22]. In the present study, we compared taurolidine with chlorhexidine digluconate which



**Fig. 2** Changes in viabilities of microorganisms by different chlorhexidine digluconate and taurolidine concentrations after 1 and 2 h. Reduction of viability is shown. That means, -100% represents a complete elimination. Changes of viability was calculated for each strain separately; the mean and standard deviation for all 32 investigated strains are presented

**Table 4** Number of strains which were totally killed after addition of the antimicrobials for 2 h

Microbial strains (number)	CHX (%)			Taurolidine (%)		
	0.005	0.02	0.08	0.1	0.4	1.6
“Real” periodontopathogens						
Gram-positive bacteria (3)	1	2	3	0	1	1
Gram-negative bacteria (14)	11	14	14	1	6	11
Potentially superinfecting						
Gram-positive bacteria (9)	4	9	9	0	4	8
Gram-negative bacteria (5)	2	4	4	0	0	5
<i>Candida albicans</i> (1)	1	1	1	0	0	0

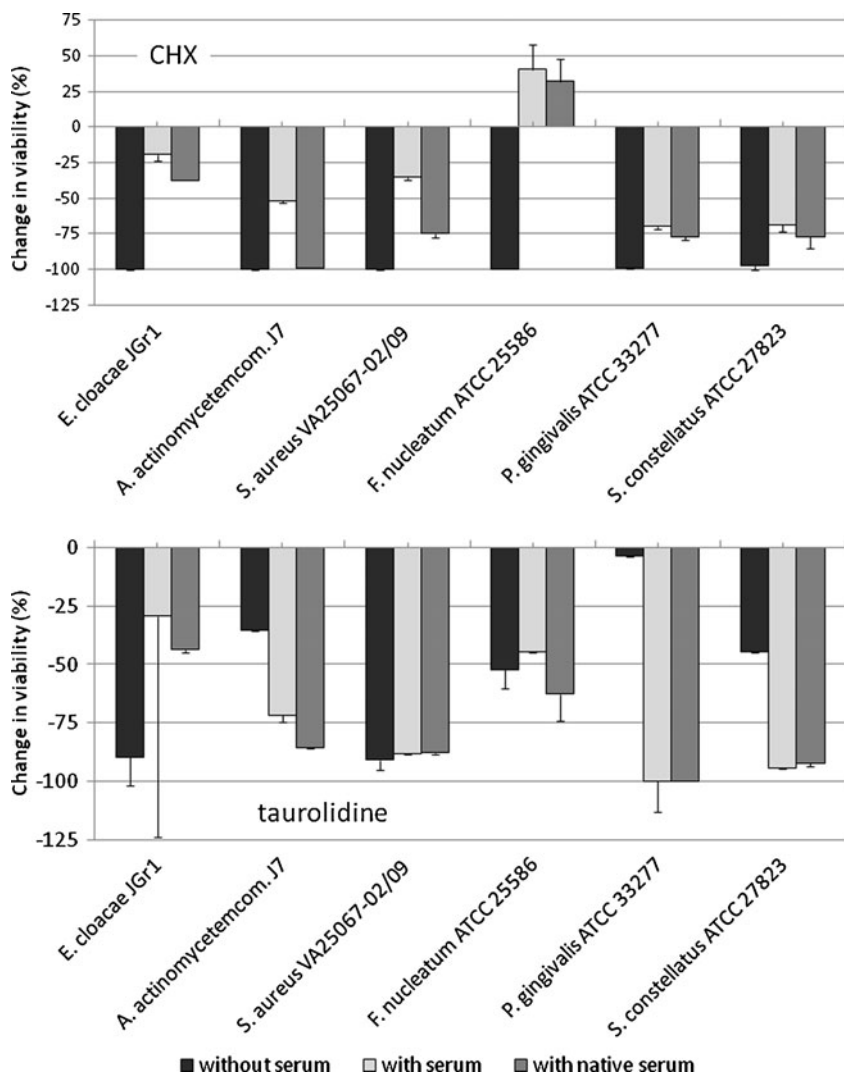
Microorganisms ( $10^4$ ) were added by taurolidine and CHX in different. After 2 h of incubation, the numbers of viable bacteria were determined by enumeration of colony forming units. The number of strains which were totally killed is listed (detection level, ten microorganisms)

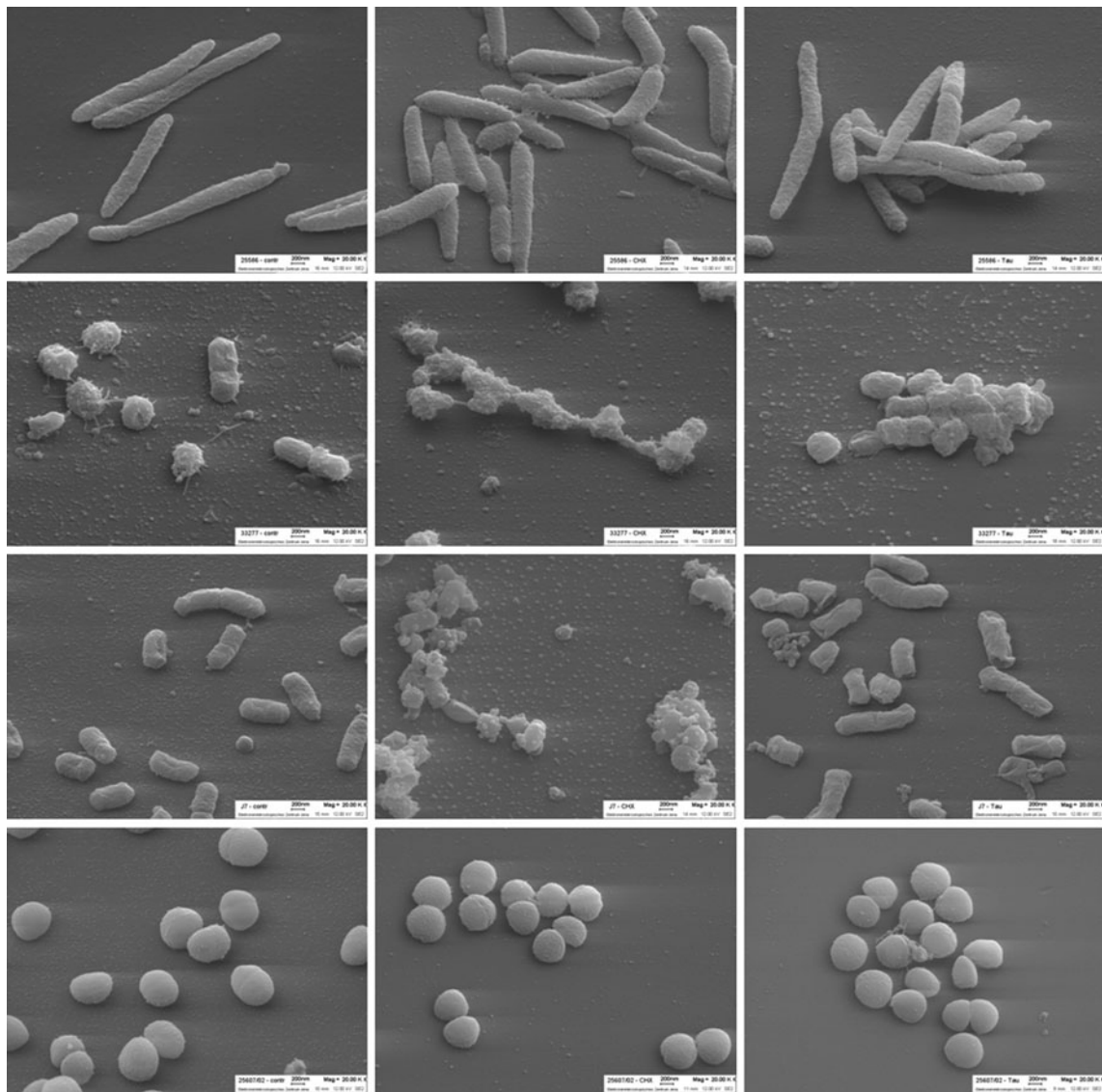
is one of the best documented antimicrobial agents in dentistry [23]. The MICs of taurolidine were all below 5% of the normally used concentration of that substance with the exception of *Candida albicans* ATCC 76615. This confirms the findings from an earlier study which determined MIC values against seven oral species; among them, one *F. nucleatum* and one *Prevotella intermedia* strain [24].

Similar to this study [24], lower MIC values were found for chlorhexidine compared to taurolidine.

With the exception of the *C. albicans* strain which was found being completely resistant to taurolidine, minimal microbiocidal concentrations of that antimicrobial were in mean one to two steps higher than the MICs. Additionally to *C. albicans*, taurolidine showed only a limited bacteri-

**Fig. 3** Influence of serum (inactivated and native) on killing rates of selected bacterial strains by 0.005% chlorhexidine digluconate and 0.1% (*P. gingivalis* 0.4%) taurolidine after 1 h. Concentrations of the disinfectants have been chosen in relation to the MIC values. Serum was added in a final concentration of 25% v/v. Comparison of native with inactivated serum shows the potential bactericidal effect of complement. Changes of viability are shown, negative values mean killing efficacy of the compounds, whereas positive values suggest a growth-promoting effect





**Fig. 4** *Fusobacterium nucleatum* ATCC 25586, *Porphyromonas gingivalis* ATCC 33277, *Aggregatibacter actinomycetemcomitans* J7, and *Staphylococcus aureus* (MRSA) VA25607-02/09 (from top to

bottom) after 1 h exposure of 0.08% chlorhexidine (middle) and 1.6% taurolidine (right) as well as a control (left)

cidal effect on *E. faecalis* ATCC 29213, which is known as the test strain for antiseptics; the MBC for that species was 0.4% being 40% of the normal concentration. Thus, the present results confirm earlier MIC and MBC values reported for taurolidine, even a larger difference between MIC and MBC values for *E. faecalis* [25].

Focusing on the included species, different aspects need to be pointed out. First of all, taurolidine was more active against the “real” periodontopathogens compared to the potentially superinfecting species. This might be attributed to the more or less incomplete activity against the included *C. albicans* strain. Secondly, taurolidine was found to be similarly active against Gram-positive and Gram-negative bacterial strains. This is in accordance with an earlier study

where aerobic and anaerobic species originated from dentoalveolar infections were tested [26]. Taurolidine is described as an unstable molecule in aqueous solution, while masked formaldehyde is released which inactivates endotoxin [27]. Furthermore, it was suggested that other mechanisms such as interaction with peptidoglycan contribute to the antimicrobial action [28]. SEM photographs of Gram-negative species appear to support an interaction with compounds of the cell wall.

In the MIC assays, the chosen highest test concentrations being in the range of about 20% of the concentrations in commercially available products were selected because of the necessity to add nutrient broth and bacterial suspension. This reflects also a general rule in pharmacology for

antibiotics that MICs should exceed the in vitro concentration by the factors 2 to 8 [29]. In the killing assays, up to 80% of the application concentration was tested. At that concentration, both taurolidine and chlorhexidine reduced the viability of the microorganisms by more than 99% after 2 h in general. Most of the used microbial strains were completely killed. But taurolidine exerted a clear concentration and time-dependent effect. Killing was enhanced after 2 h. At a lower concentration of taurolidine, the antimicrobial activity was often completely blocked. Based on the findings, it may be anticipated that a sufficient high concentration of taurolidine needs to be ensured for several hours, while a dilution of taurolidine, e.g., by the flow of gingival crevicular fluid should be avoided as much as possible. Thus, from a clinical point of view, the choice of an optimal carrier device, ensuring a long-lasting, sufficiently high concentration of the active substance, appears to be a key factor, which may significantly affect the therapeutic use. In the treatment of periodontal infections, the topical application of antibiotics incorporated in various types of controlled released devices, i.e., such as gels [30, 31] or microspheres [32] represents an important therapeutic modality.

Gingival crevicular fluid contains up to 35% of the albumin found in serum [33]. After non-surgical and surgical periodontal treatment, serum-rich wound fluid is produced. Therefore, the activity of antimicrobials in the presence of serum plays an important role. Our results clearly indicate that taurolidine was active in a serum-rich environment, while on the contrary, the efficacy of chlorhexidine was dramatically decreased. This finding may suggest that taurolidine may be also applied in a serum-rich environment, e.g., after periodontal surgery. An enhanced bactericidal activity of taurolidine in the presence of serum as described before [34] was not always found. This might be associated with a resistance of many periodontopathogens against killing by complement [35, 36]. In killing assays, both the potential inhibiting effect of serum proteins and the complement activity are combined with the efficacy of the antimicrobials. In the present study, only selected strains were tested, and it can be suggested that the complement effect does not overcome an inhibition by the other serum compounds.

Serum may also be an important component of subgingival plaque being a biofilm. The special conditions of biofilms were not addressed in this study. An earlier made study found a plaque-growth reducing effect of taurolidine which was less pronounced in comparison to chlorhexidine [37]. Recently, experiments in healthy volunteers have shown a decreased viability in supragingival plaque after 2 min of rinsing with taurolidine; again, the potential antimicrobial effect was not as high as after rinsing with chlorhexidine [38]. In that study, the bactericidal effect was determined immediately after the 2-min exposure. Con-

cluding from our results showing a time-dependent antimicrobial effect of taurolidine at a later time-point differences might be less remarkable between the two antimicrobials. A depot effect can be suggested for taurolidine, as a lock solution, prevents successful infections in catheters [39]. Biofilms are organized microbial communities surrounded by a biopolymer matrix and characterized by slow growth, increasing mutation frequency, and maximum tolerance to antibiotics [40]; the special conditions of biofilms should be addressed in subsequent studies testing taurolidine.

Pathogenesis of periodontitis comprises the microbial challenge and the host response with an inflammatory and immune response directed to cope with the challenge. The host response results in production of many proinflammatory cytokines involved in inflammation and bone loss [41]. It is well-established that periodontopathic bacteria are able to attach and to invade epithelial cells [42, 43], while polymorphonuclear neutrophils interacting with periodontopathic bacteria release reactive oxidative species [44], which in turn damage the soft and hard periodontal tissues. Taurolidine has been demonstrated to inhibit adhesion of *Escherichia coli*, *Staphylococcus saprophyticus*, and *C. albicans* to mucosal epithelial cells [3] and of Gram-negative species to plastic surfaces [39]. Taurolidine derivatives decrease production of proinflammatory cytokines and oxidants [45]. Thus, the positive immunomodulatory properties of taurolidine might be of importance in periodontitis and should be analyzed in further in vitro and in vivo studies.

Taken together, the present findings suggest that: (a) taurolidine possesses antimicrobial properties which are not reduced in the presence of serum which is a main component in gingival crevicular and wound fluid and (b) the demonstrated in vitro antimicrobial properties of taurolidine warrant further evaluation in non-surgical and surgical periodontal therapy.

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**Conflict of interest** The authors declare that they have no conflicts of interest.

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