

# Chlorhexidine – pharmaco-biological activity and application

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**Abstract. – OBJECTIVE:** Chlorhexidine (CHX) is one of the most widely used antiseptic, especially in dentistry. At low concentrations CHX is bacteriostatic and at high concentrations acts bactericidal causing cell death by cytolysis. In this study, we performed a systematic review of pharmaco-biological activity and application of CHX.

**MATERIALS AND METHODS:** Articles for inclusion in this review were retrieved from online databases PubMed/Medline. The selected papers were included in the present manuscript according to their relevance for the topic.

**RESULTS:** Totally 75 papers were enrolled in this research. CHX has strong biocidal activity against Gram-positive bacteria and weaker activity against Gram-negative bacteria. It is also active against yeasts, some dermatophytes and some lipophilic viruses. The most widely application CHX has found in dentistry and antiseptics. Numerous studies have confirmed the beneficial effects of CHX in reducing of plaque accumulation, in tooth caries, gingivitis, periodontitis and in alveolar osteitis. Unfortunately, CHX exhibits cytotoxic activity on human cells, can cause colorization of teeth and fillings, and its activity depends on the pH of the environment and the presence of organic substances.

**CONCLUSIONS:** CHX plays a valuable role in the dentistry and antiseptics. However, it can also cause side effects, limiting its application time.

*Key Words:*

Chlorhexidine, Antiseptic, Biocidal activity, Cytotoxicity, Dentistry.

## Introduction

Chlorhexidine (CHX) is one of the most commonly used antiseptic agents for skin and mucous membranes disinfection. In dentistry is used as mouth rinses, oral irrigations and slow release devices. CHX is helpful in gingivitis and periodontitis, is used as an adjunct to scaling and root planing procedures. Also for prevention of

dental caries, oropharyngeal decontamination and in endodontal treatment<sup>1,2</sup>. Moreover, it is used in hand hygiene in health-care personnel, general skin cleanser, in catheter site preparation, in bladder irrigation<sup>3,4</sup>. CHX is active against Gram-positive and Gram-negative bacteria, yeasts and viruses<sup>5,6</sup>. The aim of this article was to provide a comprehensive review regarding application and biological activity of chlorhexidine.

## Materials and Methods

The present study offers a review of the literature dealing with the chemical properties, antimicrobial activity, application and side effects of the chlorhexidine. To this effect, a Medline search was carried out, using the PubMed search. The identified papers were selected for inclusion in the present manuscript according to their relevance for the topic. The search was restricted to human subjects. We also reviewed the relevant references listed in the searched papers to identify potential related articles. Other articles and information were also identified in author's personal archive.

## Results

### Chemical Aspects

Chlorhexidine molecule is composed of two symmetrical structure with 4 chlorophenyl rings and 2 biguanide groups linked by a central hexamethylene bridge<sup>7,8</sup>. CHX is a cationic molecule and their biological activity owes single chlorine atoms bars on both phenolic rings. CHX is 1,1'-hexamethylene bis (5-[4-chlorophenyl] biguanide) having the chemical formula  $C_{22}H_{30}Cl_2N_{10}$  (<http://toxnet.nlm.nih.gov>). It is a strong alkali practically insoluble in water. In the water soluble are the salts of chlorhexidine and

they have been applied in disinfectant formulations: chlorhexidine diacetate, chlorhexidine digluconate and chlorhexidine dihydrochloride<sup>9</sup>. CHX compound most widely used in disinfectant formulations is gluconate. Chlorhexidine gluconate is almost colorless or pale yellow liquid, and is highly soluble in water. Chlorhexidine acetate is a white microcrystalline powder. The substance is very sparingly soluble in water and soluble in 96% ethanol<sup>7</sup>. Chlorhexidine digluconate solution<sup>9</sup> is an aqueous solution which contains 1,1 -(hexane-1,6-diy)bis[5-(4-chlorophenyl)biguanide]di-D-gluconate, with the chemical formula  $C_{34}H_{54}Cl_2N_{10}O_{14}$ . Chlorhexidine hydrochloride is a white to off-white crystalline powder. Substance is quite sparingly soluble in water and very difficult in 96% ethanol<sup>8</sup>. Aqueous solutions of chlorhexidine are relatively resistant to elevated temperatures. Heating to 100°C does not cause decay. During long-term storage under the influence of light and air chlorhexidine solution gradually darkens. Chlorhexidine activity is dependent on the pH of the environment, and the optimal range is 5.5-7.0. Activity is reduced in the presence of serum, blood, pus and other organic matter. Its activity is also reduced in the presence of soaps and other anionic compounds<sup>7,10</sup>.

### Mode of Action

The antimicrobial effect of chlorhexidine is dose-dependent. Chlorhexidine at low concentrations (0.02%-0.06%) has bacteriostatic activity, whereas at higher concentrations (> 0.12%) acts bactericidal (11). CHX is a cationic molecule and binds nonspecifically to negatively-charged membrane phospholipids of bacteria. At low concentrations CHX affects the change in the osmotic balance of the bacteria cell. This leads to the release of potassium, phosphorus and other low-weight molecules<sup>12</sup>. The process, taking place in an environment of sublethal concentration of chlorhexidine, leads to a loss of as much as 50% of potassium ions; it may be reversible, on condition of removing the compound<sup>7,10</sup>. At high concentrations CHX causes cell death by cytolysis. It leads to the release of the main intracellular components, including nucleotides, to changes of the cell's protein structure and precipitation/coagulation of cytoplasmic proteins<sup>13</sup>.

### Antimicrobial Activity

Chlorhexidine has a wide spectrum of antibacterial activity. The bactericidal action of CHX is

more effective against Gram-positive bacteria and weaker against Gram-negative ones<sup>14</sup>. CHX is also active against fungi and viruses<sup>5,6</sup>. Chlorhexidine is not lethal to acid-fast organisms<sup>15</sup>. It is not sporicidal, however may be sporicidal at elevated temperatures<sup>16-18</sup>. Some bacteria, e.g. strains of *Proteus* and *Providencia*, may be highly resistant to the CHX<sup>19-21</sup>. Chlorhexidine as an antiseptic shows comparable activity against *Staphylococcus aureus* strains susceptible to methicillin (MSSA) and strains resistant to methicillin (MRSA)<sup>22,23</sup>. Also in the case of both resistant to vancomycin strains of enterococci (VRE) and sensitive to vancomycin showed a comparable sensitivity to chlorhexidine<sup>24</sup>. In *ex vivo* studies have been shown effectiveness of CHX solution against *Actinomyces israelii* and *Enterococcus faecalis*<sup>25-27</sup> in infected root canal systems. Vianna et al<sup>28</sup> have investigated *in vitro* the antimicrobial activity of CHX against endodontic pathogens: *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*. CHX eliminated also anaerobic periopathogens: *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, and *Prevotella intermedia*. Agents containing chlorhexidine gluconate are effective against *Propionibacterium*, *Selenomonas* and *Serratia marcescens*<sup>14,29</sup>. Bacteriostatic activity of chlorhexidine against various microbial species is presented in Table I.

**Table I.** Bacteriostatic activity of chlorhexidine against various microorganisms (based on ref. 30-40).

Microorganism	Minimal inhibitory concentration (MIC) of chlorhexidine (µg/mL)
<i>Staphylococcus aureus</i> MSSA	0.25-8
<i>Staphylococcus aureus</i> MRSA	2-8
<i>Enterococcus faecalis</i>	4-16
<i>Streptococcus mutans</i>	0.9-4
<i>Lactobacillus reuteri</i>	0.125-4
<i>Lactobacillus fermentum</i>	0.25-1
<i>Lactobacillus acidophilus</i>	0.5-2
<i>Porphyromonas gingivalis</i>	0.9
<i>Fusobacterium nucleatum</i>	1.8
<i>Escherichia coli</i>	2-16
<i>Klebsiella</i> spp.	8-16
<i>Pseudomonas aeruginosa</i>	16-32
<i>Candida albicans</i>	1-16
<i>Candida tropicalis</i>	75
<i>Candida krusei</i>	150
<i>Aspergillus</i> spp.	8-64

It was observed a correlation between the sensitivity of microorganisms to antibiotics and susceptibility to chlorhexidine. Resistance of Gram-negative bacteria for such antibiotics, as ciprofloxacin, imipenem, cefotaxime, ceftazidime, aztreonam and gentamicin, was accompanied the increase in resistance to chlorhexidine<sup>41</sup>.

Chlorhexidine has activity against dermatophytes and yeasts. Sensitivity of fungi, both on growth inhibitory concentrations, and cidal concentrations is similar (e.g. *Candida albicans*) to the sensitivity of vegetative cells of bacteria or higher (e.g. *C. tropicalis*, *C. krusei*)<sup>5,42-44</sup>. Studies have shown that the use of mouthwash with 0.12% CHX for up to three months (with monthly interval during treatment) has both therapeutic effect in oral fungal infection and prevent the development of infections caused by *C. albicans* in HIV-infected children<sup>45</sup>. It has been observed that chlorhexidine affected *C. albicans* pathogenicity – inhibited filamentation, probably as the result of some enzyme inhibition<sup>46</sup>. Some studies have shown that yeast adhesion to epithelial cells was reduced after pretreatment of yeast with the CHX<sup>47,48</sup>.

Under the influence of CHX are rapidly inactivated lipophilic viruses (e.g. herpes simplex virus, HIV, influenza virus, cytomegalovirus). Chlorhexidine like other antiseptics has no significant virucidal activity against small non-enveloped viruses (enteroviruses, polio viruses, papilloma viruses)<sup>6</sup>. Wood and Payne demonstrated inactivating effect of CHX on the enveloped viruses herpes simplex virus type 1 and human immunodeficiency virus type 1, whilst this substance were ineffective against enveloped human coronavirus, and the non-enveloped viruses<sup>49</sup>.

### **Cytotoxic Activity to Human Cells**

Many reports describe cytotoxic effect of chlorhexidine to human gingival fibroblasts<sup>50,51</sup>, human periodontal ligament cells<sup>52</sup>, human alveolar bone cells<sup>53</sup>, and human osteoblastic cell line<sup>54</sup>, which is time- and dose-dependent. Solution of 0.02% CHX presents high cytotoxicity, and lower concentrations of CHX: 0.0024% and 0.004% cause slight cytopathic effects to the odontoblast-like cells MDPC-23<sup>55</sup>. After exposing the odontoblast-like cells MDPC-23 to the CHX solutions at concentrations of 0.06%, 0.12%, 0.2%, 1% and 2%, and times 60 s, 2 h or 60 s with a 24-h recovery period has been demonstrated decrease in cell metabolism (MTT

assay) and total protein concentration. The least cytotoxic to the cells was 60-s exposure time and the most toxic was exposure to CHX for 60 s with a 24-h recovery<sup>56</sup>. CHX also affects the change in the level of cellular ATP. Depletion of cell ATP occurs, in a time- and concentration-dependent manner, in concentrations of CHX > 0.001%. Concentrations  $\geq$  0.02% produces total loss of ATP in human dermal fibroblasts. At the same time, CHX concentrations  $\geq$  0.005% are required to produce total cell death<sup>12</sup>. Giannelli et al<sup>54</sup> showed that chlorhexidine is able in different cell types *in vitro* to cause alterations in actin cytoskeletal assembly, alter mitochondrial membrane potential, trigger intracellular Ca<sup>2+</sup> increase and cause reactive oxygen species generation, and stimulate apoptosis and autophagic/necrotic cell death.

### **Application of Chlorhexidine**

It has been shown that CHX is very effective as surgical hand antiseptic, to destroy transient microorganisms and inhibit the growth of resident microorganisms<sup>4,57</sup>. Preoperative skin preparation with 0.5% chlorhexidine in methylated spirits was associated with lower rates of surgical site infections (SSIs)<sup>58</sup>. Rubin et al<sup>59</sup> indicated that the intervention of bathing with CHX can reduce the number of hospital-acquired infections (HAIs). The reduction in HAIs using CHX was found to be greater as compared to bathing with soap and water. CHX is also used in obstetrics and gynecology. There is significant evidence that topical application of chlorhexidine to umbilical cord reduces neonatal mortality<sup>60</sup>. Simultaneously, CHX can be used for prevention of infection following caesarean section<sup>61</sup>.

The most widely application CHX has found in dentistry. CHX is available as oral rinses, aerosols and spray formulations (0.12-0.2%), gels (0.12-1%) and dental varnishes (1%, 10%, 40%). CHX also can be found in toothpastes, gels for cleaning teeth and dental flosses<sup>13,62</sup>. Using of 0.12-0.2% concentrations of CHX in mouthwashes results in significant reduction of gingival inflammation and plaque indexes<sup>63</sup>. The chlorhexidine as well as chlorhexidine-sodium fluoride mouthwashes have a significant effect on inhibition of plaque accumulation and gingivitis<sup>64-67</sup>. Also, the application of chlorhexidine varnish have beneficial effects in gingivitis, reducing plaque accumulation, bleeding levels and gingival index<sup>68,69</sup>. Using of 0.12% chlorhexidine rinsing pre-operatively and 7 days postop-

eratively, have significant and clinically relevant preventive effect on alveolar osteitis (dry socket) following surgical removal of lower third molars<sup>70</sup>. CHX is used also in prevention of tooth caries. It has inhibitory effect on the formation of dental plaque and acts against different species of *Streptococcus*, including *S. mutans* – main etiological agent of tooth caries<sup>71,72</sup>. Solutions containing different concentrations of CHX have been suggested for the irrigation of infected root canals<sup>2,8,73</sup>. CHX can also have impact in the treatment of halitosis, especially in reducing the levels of anaerobic bacteria related with halitosis<sup>74</sup>.

Chlorhexidine is considered to be a effective drug in dentistry<sup>6</sup>, but used for a long time (more than 2 weeks) in dental treatment most often causes discoloration of the teeth, tongue and fillings made of composite materials and glass-ionomer. Moreover, CHX can cause mucous membrane irritation and taste disturbance, but these symptoms are transient and disappear following termination of therapy<sup>64,66,75</sup>.

## Conclusions

Chlorhexidine is a cationic antiseptic. It is characterized by a wide range of antimicrobial activity, against Gram-positive and Gram-negative bacteria, some yeasts and some viruses. This antiseptic is widely used, especially in the prevention and treatment of the oral cavity diseases. CHX has a small number of side effects *in vivo*, including teeth and fillings colorization. However, more important is cytotoxic activity to human cells *in vitro*, which can stimulate apoptosis and autophagic/necrotic cell death. Cytotoxic activity of CHX to human cells *in vivo* is recently not fully known and requires further studies.

## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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