

## ABSTRACT

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**Title:** Evaluation of antimicrobial effectiveness of octenidine derivatives

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**Introduction:** Octenidine dihydrochloride belongs to the group of so-called "gemini" surfactants, or bis-quaternary equivalents of quaternary ammonium salts (QAS). QAS are generally widely used disinfectants exhibiting relatively low toxicity and high efficacy. Bis-quaternary QAS are characterised by even better surface activity and biodegradability. Octenidine dihydrochloride is considered as a very promising antimicrobial agent for its high antimicrobial activity even against multi-drug resistant bacteria. However, its limited solubility in aqueous solvents complicates its application due to the need of organic solvents in product composition, which afterwards reduce wounds or mucous membranes tolerability.

**Aim:** The aim of this work was to test the antimicrobial efficacy of 11 new derivatives of octenidine dihydrochloride, which were prepared with emphasis to increase efficiency and improve solubility in aqueous solutions.

**Methods:** The microdilution broth method was used in the first phase of testing to determine the basic antimicrobial parameters – minimum inhibitory and bactericidal concentrations - for all 11 compounds and octenidine dihydrochloride as reference compound. Measurements were performed on 4 Gram-positive bacterial strains (*Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, Vancomycin-resistant *Enterococcus*) and 4 Gram-negative bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella pneumoniae* producing extended spectrum  $\beta$ -lactamases (ESBL), multidrug-resistant *Pseudomonas aeruginosa*). Furthermore, the minimum biofilm eradicating concentration was

determined for the 7 most effective new compounds (plus octenidine dihydrochloride) on the *Staphylococcus aureus* biofilm by using the Calgary biofilm device.

**Results:** Two substances were excluded from further testing after the first measurement due to their low solubility and antimicrobial activity. The remaining nine compounds showed excellent activity against Gram-positive bacteria, and four of them showed similarly excellent activity against Gram-negative bacteria. The highest activity was observed on derivatives with alkyl side chains composed of 8 or 10 carbons and a centre linker of 8 or 10 carbons. Compounds that underwent antibiofilm activity determinations demonstrated a significant ability to eradicate a grown biofilm.

**Conclusion:** Antimicrobial activity has been established for all newly prepared octenidine dihydrochloride derivatives. Several derivatives have shown the same or higher antimicrobial activity as the reference agent octenidine dihydrochloride, including the ability to eradicate bacterial biofilm.

**Key words:** “gemini” surfactants, octenidine dihydrochloride, antimicrobial efficacy testing, microdilution broth method, minimum inhibitory concentration, minimum bactericidal concentration, minimum biofilm eradicating concentration