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Efficacy of Sodium Hypochlorite (NaOCl) Against Gram Positive and Gram Negative Bacteria: A Systematic Literature Review

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ABSTRACT: Sodium hypochlorite (NaOCl) is a chemical compound that acts as a strong oxidizer commonly used as an antibacterial, antiseptic, and disinfectant in various pharmaceutical and health applications. This systematic review aims to evaluate the antibacterial activity of NaOCl against various types of bacteria based on the PICO framework. Literature searches were conducted through the PubMed, ScienceDirect, and Scopus databases for publications from 2017 to 2024. Inclusion criteria included all types of bacteria, the use of NaOCl as an antibacterial or antimicrobial agent, and articles published within that time frame. The interventions reviewed were the use of NaOCl at various concentrations, while the control group referred to conditions without treatment or a comparative antibacterial agent. The results were determined based on antimicrobial activity against the bacteria tested. Two stages of independent literature searches were conducted according to the established inclusion and exclusion criteria. This review shows that NaOCl has significant antibacterial activity against various bacteria, with effectiveness depending on the concentration and characteristics of the target microorganisms.

Keywords: Sodium Hypochlorite; Antibacterial,; Gram-Positive Bacteria; Gram-Negative Bacteria

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INTRODUCTION

Sodium Hypochlorite (NaOCl) is a chemical compound that has been widely used since 1936 by Walker primarily as a powerful antimicrobial agent (Luddin & Aly Ahmed, 2013). NaOCl is basically a broad-spectrum antimicrobial compound in the form of an aqueous liquid and is able to inhibit the growth of various types of bacteria, viruses, fungi, and even spores that are very strong. The availability of NaOCl in various disinfectant products is very easy to find. NaOCl has been reported to have antibacterial activity on *Escherichia coli, Enterococcus faecalis, Strepococcus aureus, Staphylococcus epidermidis* and many more (Luddin & Aly Ahmed, 2013; Sulistya, 2020). NaOCl can even overcome the influenza virus that causes flu and the coronavirus that causes Covid-19 so it is used as a disinfectant (Lachenmeier, 2021). The usefulness of NaOCl as a broad-spectrum antimicrobial makes the compound commonly used in disinfectant products. The mechanism of antibacterial action of NaOCl generally occurs through the oxidation process. When NaOCl interacts with microbes, hypochlorous acid (HOCl) can be produced which gives it properties as a strong oxidizing agent. The presence of hypochlorous acid (HOCl) is able to damage cell membranes, protein structures, lipids, and other important molecules in a microbe which triggers cell damage and death in the microbe (Boecker et al., 2023). As a strong oxidizing agent, NaOCl can exert toxic effects and corrosive properties. At high concentrations, toxic effects such as burning sensation and eye damage may occur when NaOCl comes into direct contact with mucous membranes and skin surfaces (Boecker et al., 2023; Marlita et al., 2024).

Apart from being used as an antimicrobial agent in disinfectant and clothes whitening products, NaOCl can also still be found in certain limits in cosmetic preparations. One of the cosmetic products that are still widely circulated in the community is cosmetic products in the form of creams such as facial brightening creams. In general, facial brightening creams work by removing dark spots or can be used in changing skin color which usually consists of a mixture of chemicals with other ingredients such as sunscreen (Bragzzi et al., 2019; Indriaty et al., 2018). Sunscreen ingredients commonly used as active substances in whitening/lightening creams are hydroquinone, whose use is currently banned because it can cause dependence and irritation on facial skin. NaOCl is still allowed as an additional ingredient in face lightening cream preparations either as an active substance as an antibacterial and/or as a preservative. In general, the addition of active ingredients that function as antibacterials in cosmetic preparations including anti-acne creams is still allowed (Marlita et al., 2024). The indication of the active ingredient NaOCl in the product is often said to have effectiveness as an anti-acne because of its ability to inhibit *Propionibacterium acnes* bacteria. The addition of active ingredients that claim to be anti-acne is necessary to provide the right indication for facial problems such as acne. However, not all ingredients that have antibacterial or antimicrobial properties are allowed to be used in cosmetic preparations, especially anti-acne.

Propionibacterium acnes is one of the bacteria that causes many acne problems through the mechanism of forming chronic inflammation in the polysebaceous glands of the skin which can aggravate acne conditions. This bacterium is a gram-positive bacterium with the characteristics of not having spores and being able to survive on human skin. Acne affects the sebaceous follicles found on the face, chest and shoulders. One of the properties of bacteria is that they can stick to surfaces and can quickly form biofilms. Biofilm is defined as a collection of microorganisms from one or more species (Reyhani et al., 2017).

Therefore, the content of active ingredients as antibacterials in the manufacture of creams must indeed be considered for use with the aim of providing good indications according to the problems that arise on the face. As previously described, Sodium Hypochlorite (NaOCl) is an ingredient that can be used as an antibacterial, antiseptic and disinfectant in various medical and occupational settings, as well as in certain cosmetic products and household uses. An important element in this compound is that it has immunologic effects (contact dermatitis) after topical use (Lachenmeier, 2021).

This systematic literature review aims to evaluate the efficacy of Sodium Hypochlorite (NaOCl) compounds against gram-positive and gram-negative bacteria commonly contained in disinfectants, bleaches, and facial brightening creams as antimicrobials, especially antibacterials that have not been done in other articles before.

METHODS

Inclusion criteria

The inclusion criteria used in this systematic review are Sodium Hypochlorite (NaOCl) compounds, articles from 2017 to 2024, NaOCl as antibacterial or antimicrobial, all types of bacteria, all countries, and original articles in vitro in English. Exclusion criteria in this study were articles in the form of reviews, theses, dissertations, and posters.

Information sources and searches

The electronic databases used in the information search were from 3 databases including PubMed, Sciencedirect, and Scopus. The keywords used in the literature search were: (Sodium hypochlorite) AND (Antibacterial OR antimicrobial activities) AND (gram-positive bacteria) AND (gram-negative bacteria).

Study Selection

The results of the information search based on the keywords used in the 3 different databases were collected together. Then all the studies obtained were selected through a preliminary process of duplication checking. Duplication checking was checked manually and duplicate citations were coded by (author name, year, and title) to create a reference data set. The purpose of duplication checking was to avoid errors and mistakes in the subsequent screening process. The next step was to screen the titles and abstracts based on the inclusion criteria set previously. Titles and abstracts that did not meet the inclusion criteria were deleted. After that, full-text articles were downloaded to be screened based on the inclusion criteria in order to obtain results in accordance with the objectives. Thus, several articles were obtained that fit and met the predetermined inclusion criteria.

Data collection and data items

Data extraction from articles obtained according to eligibility (inclusion) is organized using tables to make it easier to extract data according to the items used. The table used contained the following information: author and year of publication, country, purpose of the study, sample used.

RESULT AND DISCUSSION

A review of the efficacy of Sodium Hypochlorite (NaOCl) against gram-positive and gram-negative bacteria has been conducted with this systematic literature review. Figure 1 below summarizes the search strategy process in the databases used. Initial searches were obtained from 3 databases namely PubMed (68), Sciencedirect (351) and Scopus (34).

So that the total number obtained is 453 articles from the range of 2017 to 2024. Of the 453 articles after screening the titles and abstracts, 365 were not suitable and resulted in 80 articles. Then from 80, articles in the form of reviews, case studies, research were excluded and obtained 8 articles according to the inclusion criteria that will be reviewed.

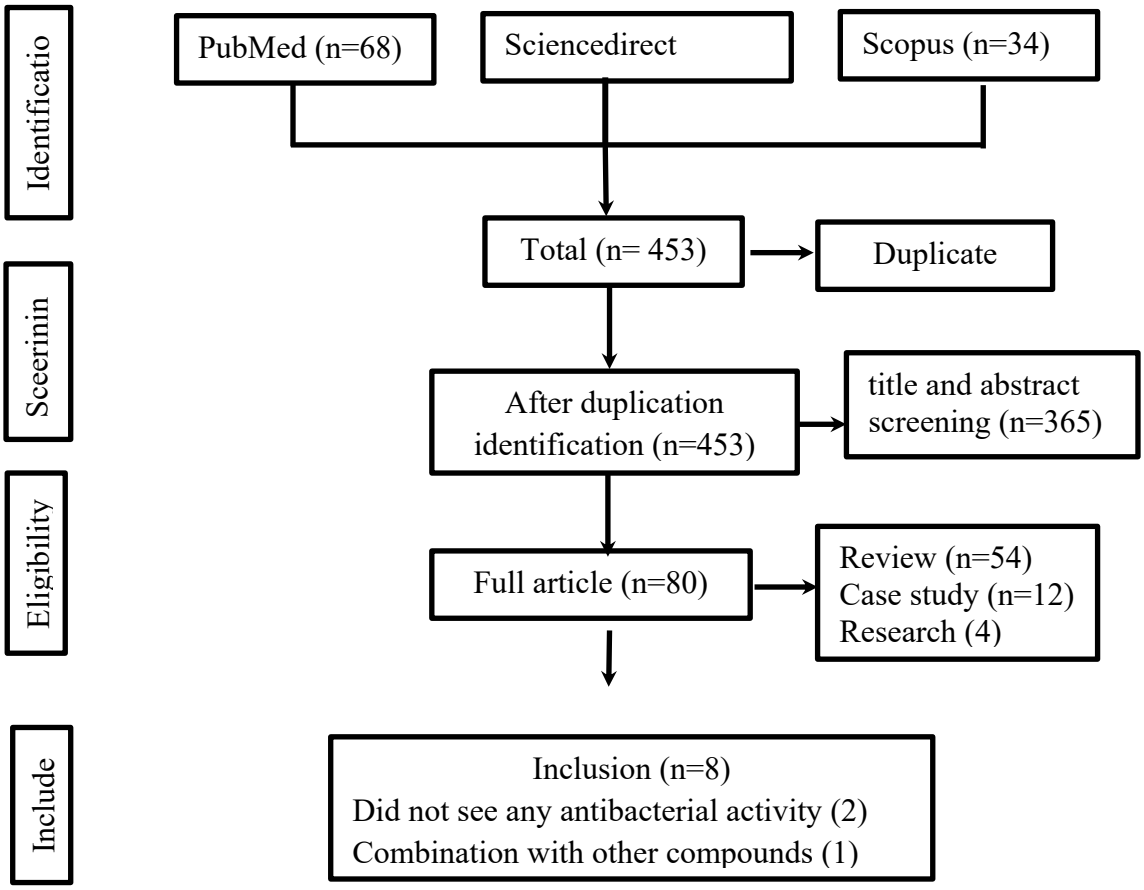

Figure 1 Literature search and selection process for review

Tabel 1. Antibacterial activity of sodium hypochlorite

Literature	Country	Purpose	Sample	Bacteria	Results
Alanso et al., 2017	Spain	To evaluate the antibacterial effect of photodynamic therapy (PDT), chlorhexidine (CHX) 2%, triantibiotic mixture (TAM), propolis, and ozone on root canals. Tooth contaminated with 0.1 mL *Enterococcus faecalis*	Photodynamic therapy (PDT), Chlorhexidine 2%, Triantibiotic mixture (TAM), propolis, ozone, NaOCl 2.5%,	*Enterococcus faecalis*	The groups treated with PDT, 2% CHX, TAM, propolis and ozone were able to show similar antibacterial potential with 2.5% NaOCl. However, the treatment group with NaOCl 2.5% based on SEM analysis showed the lowest percentage of contamination
Köhler et al., 2018	Germany	Determining the efficacy of sodium hypochlorite (NaOCl) against multidrug-resistant gram-negative bacteria (MDR-GNB) using the macrodilution method	NaOCl 0.1% and 0.2%	*Ainetobacter baumani, Pseudomonas aeruginosa, Klebsiella* spp	Quantitatively, *Pseudomonas aeruginosa* was significantly less susceptible to other bacterial strains. The *Klebsiella* genus was also significantly less susceptible (p= 0.0124).
Chum et al., 2019	Malaysia	The present study compared the antimicrobial efficacy of octenidine dihydrochloride (OCT) with chlorhexidine (CHX) and sodium hypochlorite (NaOCl) against *Staphylococcus epidermidis* (*S. epidermidis*) for root canal disinfection.	2% CHX, 3% NaOCl, and 0.1% OCT	*Staphylococcus epidermidis*	3% NaOCl showed the largest zone of inhibition among all irrigants tested ($p<0.05$). There was no significant difference between Clony Forming Unit (CFU) measurements from 2% CHX, 3% NaOCl, and 0.1% OCT indicating complete elimination of *S. epidermidis* in all samples

Literature	Country	Purpose	Sample	Bacteria	Results
Yanling et al., 2018	China	The aim of this study was to evaluate the antimicrobial effect of relacin combined with sodium hypochlorite (NaOCl) on *Enterococcus faecalis* biofilm and to evaluate the cytotoxicity of relacin.	NaOCl (0.01%, 0.05%, 0.25%)	*Enterococcus faecalis*	Combined treatment of relacin (14 mM) and NaOCl resulted in significantly higher treatment efficacy than NaOCl treatment alone Combination of relacin with low-concentration NaOCl is effective and not cytotoxic.
Reyhani et al., 2017	Iran	Evaluate the effectiveness of different concentrations of sodium hypochlorite (NaOCl) solution in reducing bacterial growth in *Enterococcus faecalis* biofilm in root canals	NaOCl 1%, 2.5%, and 5.25 %	*Enterococcus faecalis*	In groups 2 (2,5% NaOCl solution) and 3 (5.25% NaOCl solution), there was no bacterial growth due to the complete removal of *E. faecalis* biofilms, whilw the bacterial counts in group 1 (1% NaOCl solution) at 4, 6 and 10 week intervals decresed compred to the control group (PBS solution)
Mohmmed et al., 2017	England	Determine the effect of agitation of 2.5% (wt/vol) sodium hypochlorite (NaOCl) on the removal, killing, and degradation of *Enterococcus faecalis* biofilms	NaOCl 2.5%	*Enterococcus faecalis*	Reduction in the amount of biofilm achieved with active irrigation groups

Literature	Country	Purpose	Sample	Bacteria	Results
Ros et al., 2021	Spain	Assess the antibacterial effectiveness of two concentrations of sodium hypochlorite (NaOCl) (2.5% and 5.25%) activated via two techniques, passive ultrasonic irrigation (PUI) and XP-endo.®Finisher (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) (XPF) against bacterial growth in intracanal adult *Entrococcus faecalis* biofilms.	NaOCl 2.5% and 5.25%	-	NaOCl was effective in biofilm removal for all experimental groups (p>0.05)
Tsai et al., 2024	Taiwan	Evaluate the cytotoxic properties of hypochlorous acid (HOCl) in vitro and antibacterial activity at several different concentrations of HOCl and compared to sodium hypochlorite (NaOCl) and chlorhexidine (CHX) as positive controls	HOCl were made into concentration series of 100, 200, 300, 400, and 500 ppm. Positive controls commonly used antiseptic NaOCl 1.5%, CHX 0.2%	Gram negatif (*A. actinomycetemcomitans* and *P. gingivalis)* Gram positif (*S. mutans* and *S. sanguinis*)	HOCl has antibacterial activity that is concentration dependent dose but NaOCl at a concentration of 1.5% can kill all the bacteria used, NaOCl has stronger antibacterial properties than HOCl and CHX at all concentrations (p<0.05)

Table 1 above illustrates the characteristics of each study from various countries and based on the objectives to be achieved. The concentrations used also vary. The study summarized in this systematic review is that the proof of the antibacterial efficacy of Sodium Hypochlorite (NaOCl) compounds has been carried out by several countries such as: Iran, Spain, England, Malaysia, United States, Germany, and Taiwan. Not rare either the studies were to compare NaOCl with other compounds or use NaOCl as a positive control (comparison).

Antibacterial Activity of NaOCl

Sodium hypochlorite is a compound that plays a role in reducing bacterial proliferation. The most common bacteria used in testing the antibacterial activity or efficacy of Sodium Hypochlorite (NaOCl) are *Enterococcus faecalis, Staphylococcus epidermidis*, and *Staphylococcus aureus*. These three types of bacteria are Gram-positive. *Enterococcus faecalis* and its biofilm form are the most common bacterial species isolated from failed root canals and periradicular infections. Bacteria in their biofilm form according to Mohmmed et al., (2017) is structurally able to resist antibacterial agents through three mechanisms, its thick biofilm structure so that antimicrobial agents are unable to penetrate microorganisms, under the influence of small concentrations and nutrient sources resulting in slow-growing and long-lasting cells, and its biofilm can undergo phenotypic changes resulting in the proliferation of resistant phenotypes.

NaOCl Levels and Dosage Forms

Based on the literature table, Sodium Hypochlorite (NaOCl) is used in a very wide range of concentrations from 0.01% to 5.25% depending on the research objective and the type of bacteria targeted. Very low concentrations (0.01–0.25%) were found in the study by Yanling et al. (2018), where NaOCl was used in combination with relacin to increase its effectiveness against *E. faecalis* biofilm, indicating that low NaOCl can be effective when combined with other adjuvant agents.

In a study of multidrug-resistant Gram-negative bacteria, Köhler et al. (2018) used a low concentration of 0.1–0.2% but still obtained a significant antibacterial effect, indicating that even diluted NaOCl still has bactericidal potential. Meanwhile, concentrations of 1–3% are widely used in endodontic and oral disinfection contexts, such as in a study conducted by Tsai et al. (2024) in which 1.5% NaOCl was able to kill all Gram-positive and Gram-negative bacteria tested, demonstrating the effectiveness of medium concentrations for general clinical applications. High concentrations of 2.5–5.25% are widely used in E. faecalis biofilm studies, such as those conducted by (Mohmmed et al., 2017; Reyhani et al., 2017; Ros et al., 2021). This is because high concentrations are required for penetration and disruption of thick biofilms in dental root canals. In terms of dosage form, all studies used NaOCl solution (liquid form), either as an endodontic irrigant, a macrodilution test solution, or an antiseptic control. There was no use of gel or foam forms in the table, so it can be concluded that NaOCl solution remains the standard dosage form in research and clinical applications according to the table data.

Efficacy of Sodium Hypochlorite

The efficacy of NaOCl in the table can be seen from its ability to eliminate bacteria, reduce the amount of biofilm, and its advantages over other antibacterial agents. In general, NaOCl shows the highest efficacy when used at a concentration of ≥2.5%, especially in studies focusing on intracanal infections. Reyhani et al. (2017) demonstrated that 2.5% and 5.25% NaOCl were able to completely eradicate E. faecalis biofilm, while a 1% concentration only provided partial reduction, indicating a direct relationship between

concentration and antibacterial efficacy. This efficacy was further reinforced by Mohmmed et al. (2017), who reported that active irrigation using 2.5% NaOCl enhanced NaOCl's ability to damage and remove biofilms, indicating that activation techniques also influence the efficacy of this agent. In the context of antiseptic comparisons, NaOCl was also proven to be more effective than CHX, OCT, and HOCl. Chum et al. (2019) showed that 3% NaOCl produced the largest inhibition zone against S. epidermidis, while a study by Tsai et al. (2024) confirmed that 1.5% NaOCl was more effective in killing all test bacteria than 0.2% CHX and HOCl up to 500 ppm. Against multidrug-resistant Gram-negative bacteria, low concentrations of 0.1–0.2% NaOCl still showed significant efficacy (Köhler et al., 2018), reinforcing the evidence that NaOCl has a broad spectrum of action and high bactericidal capacity even against resistant pathogens. Overall, the efficacy of NaOCl in the table shows that this compound is a highly effective antibacterial agent, with the ability to kill bacteria, disrupt biofilms, and work at various concentrations and against various types of bacteria.

Sodium hypochlorite (NaOCl) efficacy is highly dependent on the volume and concentration of the solution used. In addition, the duration of irrigation indicates the time of NaOCl use which can affect its antibacterial activity. In fact, the concentration of the solution is often modified in conducting antibacterial activity tests. The higher the concentration of NaOCl used, the greater the antibacterial activity. In some conditions, if the concentration of NaOCl is too large, it can cause toxic effects in treatment. However, this side effect is not too much explained in the literature used. Other effects arising from the use of NaOCl has been done by Tejada et al. (2019) which states that the administration of NaOCl can reduce the regeneration of tissues at inappropriate concentrations.

CONCLUSION

Based on the results of a systematic review that has been carried out with inclusion criteria, Sodium Hypochlorite (NaOCl) at a concentration of 2.5% shows optimal antibacterial efficacy on gram-positive and gram-negative bacteria.

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