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Systematic Review

Antibacterial Activity of Endodontic Gutta-Percha—A Systematic Review

Jakub Kowalski [1,2], Joanna Rygas [3], Karolina Homa [4,5], Wojciech Dobrzyński [6], Rafal J. Wiglusz [2,7], Jacek Matys [8,*] and Maciej Dobrzyński [5]

1 Dental Practice, Dental Focus, Babiogórska 34, 43-400 Bielsko-Biała, Poland; drjakubkowalski@icloud.com
2 Institute of Low Temperature and Structure Research, Polish Academy of Sciences, Okolna 2, 50-422 Wroclaw, Poland; r.wiglusz@intibs.pl
3 Dental Practice, Wojciecha z Brudzewa 10, 51-601 Wroclaw, Poland; joanna.rygas@gmail.com
4 NZOZ Medident, Żeromskiego 2A, 43-230 Goczałkowice-Zdrój, Poland; kakahoma@poczta.onet.pl
5 Department of Pediatric Dentistry and Preclinical Dentistry, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; maciej.dobrzynski@umw.edu.pl
6 Department of Dentofacial Orthopedics and Orthodontics, Division of Facial Abnormalities, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland; wojt.dobrzynski@wp.pl
7 Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Krzywoustego 4, 44-100 Gliwice, Poland
8 Oral Surgery Department, Wroclaw Medical University, Krakowska 26, 50-425 Wroclaw, Poland
* Correspondence: jacek.matys@umw.edu.pl

Abstract: Numerous failures in root canal treatment (RCT), attributed to the persistence of adverse microbiota, prompted researchers to develop a biomaterial with effective antibacterial and antifungal properties. In our systematic review, emphasis was placed on examining the antimicrobial properties of gutta-percha, the most used material for root canal obturation. The review aimed to determine whether gutta-percha demonstrated adequate antibacterial and antifungal features. Additionally, it sought to identify specific substances added to gutta-percha's composition that could enhance the success rate of root canal obturation. On October 30, 2023, electronic searches were systematically performed in the PubMed, Web of Science (WoS), and Scopus databases using the specified keywords: ((antibacterial) OR (antimicrobial)) AND ((gutta-percha) OR guttapercha)). A thorough evaluation commenced, with an initial pool of 330 studies, from which 174 duplicates were methodically identified and removed. The ultimate dataset for qualitative synthesis consisted of 26 studies. The results of the compared studies did not unequivocally indicate whether the use of gutta-percha alone exhibits antibacterial or antifungal effects. Among the six studies demonstrating results supporting gutta-percha's antimicrobial activity, five showed activity against *Enterococcus faecalis*. Conversely, six studies concluded that gutta-percha lacks any discernible antimicrobial features. One study even suggested that gutta-percha might promote the progression of bacterial development. However, eight out of nine studies demonstrated an increase in gutta-percha's antimicrobial properties after the addition of chlorhexidine. Furthermore, calcium hydroxide and iodoform also improved gutta-percha's properties, showing promising results. Unfortunately, none of the materials added to gutta-percha displayed comprehensive improvement in its antimicrobial efficacy, including activity against *Enterococcus faecalis*. The review demonstrated the benefit of enhancing gutta-percha with chlorhexidine, calcium hydroxide, and iodoform. Nevertheless, achieving the inactivation of *Enterococcus faecalis* posed a challenge.

Keywords: antimicrobial; canal treatment; endodontic; *Enterococcus faecalis;* RCT

Citation: Kowalski, J.; Rygas, J.; Homa, K.; Dobrzyński, W.; Wiglusz, R.J.; Matys, J.; Dobrzyński, M. Antibacterial Activity of Endodontic Gutta-Percha—A Systematic Review. *Appl. Sci.* **2024**, *14*, 388. https://doi.org/10.3390/app14010388

Academic Editor: Dorina Lauritano

Received: 11 December 2023
Revised: 27 December 2023
Accepted: 30 December 2023
Published: 31 December 2023

1. Introduction

Bacteria and their toxins play a significant role in the etiology and progression of pulpal and periapical inflammation [1–8]. Root canal therapy becomes necessary when the defense mechanisms of the vital dental pulp are compromised, leading to the removal of

infected necrotic tissue [9,10]. The primary objective of endodontic treatment is to eliminate microbial pathogens and prevent reinfection of the canal [1–3,5–9,11–17]. Another important stage of endodontic treatment is root canal obturation, which aims to establish a hermetic seal in the endodontic structures, thereby maintaining the aseptic environment achieved during the earlier stages of root canal treatment [8]. Root canal therapy is a crucial measure for preserving teeth and avoiding their extraction [10,11,18]. Advancements in technology, a deeper understanding of root canal anatomy, and the use of improved biocompatible materials have collectively contributed to the increased success rate of root canal therapy [11]. Dental materials selected for root canal filling play a pivotal role in the success of endodontic treatments. The ideal characteristics of these materials encompass not only effective antibacterial and antifungal properties but also the ability to establish and maintain a durable antiseptic environment within the root canal system [5]. Achieving dimensional stability over time is paramount to ensure the long-term integrity of the root canal filling. Moreover, biocompatibility with oral tissues is crucial to mitigate potential adverse reactions and promote favorable healing responses. In addition to these fundamental properties, obturation materials must possess optimal radiopacity for clear visibility on radiographs [6]. Adequate radiopacity is indispensable for distinguishing between anatomical structures visible on radiographs, facilitating precise assessment of treatment quality and ease of manipulation within the intricate root canal anatomy. This radiographic clarity is instrumental in evaluating the adaptation and placement of obturation materials, ensuring a thorough and homogenous fill. These considerations collectively contribute to the overall quality of proper obturation, a critical factor in the long-term success of endodontic therapy [1,2,5,8,9,11]. Importantly, these materials should not induce irritation in periapical tissues [19] (see Figure 1).

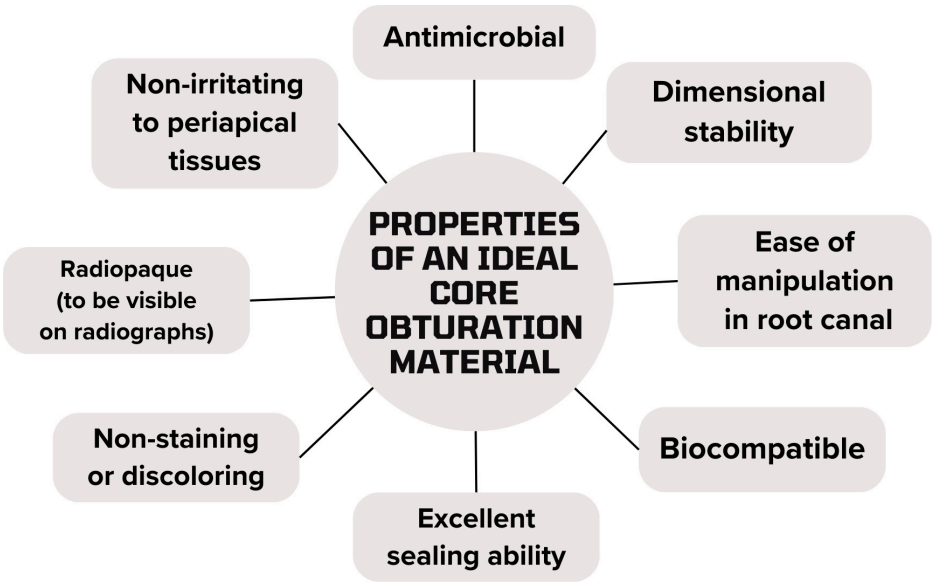

Figure 1. Figure depicts properties of an ideal core obturation material (created using Canva.com).

The failure of root canal therapy is typically attributed to reinfection of the root canal space after root canal obturation [3,4,9,11]. The intricate root canal anatomy, characterized by fins, isthmi, ramifications, and dentinal tubules, makes complete disinfection of the root canal system challenging, if not nearly impossible [20,21]. Bacteria persisting in the root canal following chemomechanical preparation may lead to the failure of endodontic treatment [4,9,20]. *Enterococcus faecalis* (*E. faecalis*) is the most isolated species recovered from infected canals [3,6,8,9,11,15,21,22]. It demonstrates resilience in extreme environments and is particularly resistant to many conventional antimicrobial agents routinely used [3,23]. *E. faecalis* is frequently identified in cases of root canal failure [3,6,8,11,18,20–22]. While *E. faecalis* is commonly associated with failed root canal therapy, other microorganisms are

implicated in endodontic treatment failure [5,9,21]. Yeasts, such as *Candida albicans*, have also been linked to endodontic failures [5,6,21]. Additionally, *Staphylococcus* spp. is one of the most common microorganisms found in stored gutta-percha cones after improper handling. Therefore, minimizing residual bacterial and fungal contamination is crucial for a successful endodontic approach [20].

Various materials have been employed in endodontics as root canal fillings, with gutta-percha being one of the most widely used for over a century [2,5,11,23]. It remains the material of choice [5,8,11] due to its biocompatibility, cost-effectiveness, long clinical usage, and potential antimicrobial properties, particularly attributed to its zinc oxide (ZnO) component [8]. Gutta-percha endodontic filling points typically consist of approximately 20% gutta-percha (matrix), 66% zinc oxide (filler), 11% heavy metal sulfates (radiopacifier), and 3% waxes and/or resins (plasticizer) [24]. However, the antibacterial effect of gutta-percha is insufficient for disinfecting root canals contaminated with common endodontic pathogens, including *E. faecalis* [5,18]. Various attempts have been made to enhance the antimicrobial and antifungal efficacy of gutta-percha (GP) points, exclusively used as an inter-appointment intracanal dressing. These efforts involve incorporating substances such as calcium hydroxide, chlorhexidine, tetracycline, iodoform, the combination of ofloxacin–ornidazole, cetylpyridinium chloride (CPC), or nanosized silver particles [2–6,8,10,11,13–18,21,25–27] (Figure 2).

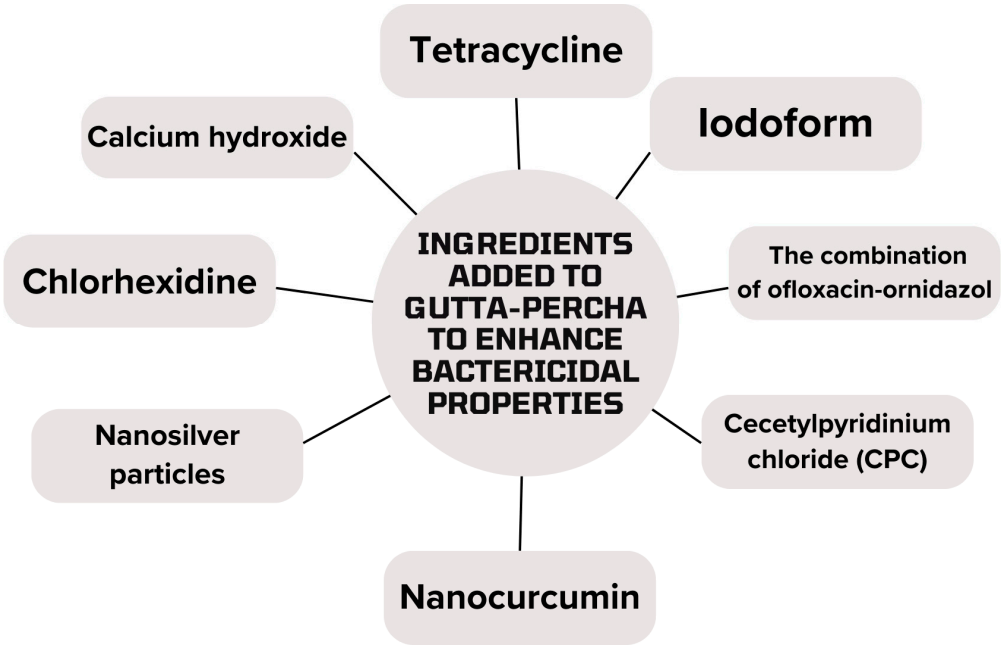

Figure 2. Figure shows ingredients added to endodontic gutta-percha to enhance antibacterial properties (created using Canva.com).

The main objective of this systematic review was to explore the impact of antimicrobial agents added to gutta-percha cones on their antimicrobial properties. Based on the analysis of articles concerning the use of various ingredients added to gutta-percha to enhance its bactericidal properties, it was concluded that, given the significance of the topic related to endodontic treatment, a systematic review on this subject is warranted. Moreover, a systematic review on this topic has not been published to date. Conducting such a literature review may motivate researchers to pursue additional studies, potentially providing substantial benefits to both endodontists and patients in the future.

2. Materials and Methods

2.1. Focused Question

This systematic review followed the PICO framework as follows.

PICO question: In the case of gutta-percha (population), will the addition of other ingredients (investigated condition) lead to a change in its antibacterial properties (outcome) compared to conventional gutta-percha without the addition of other components (comparison condition)?

2.2. Protocol

The selection process for articles in this systematic review was carefully outlined following the PRISMA flow diagram (Figure 3).

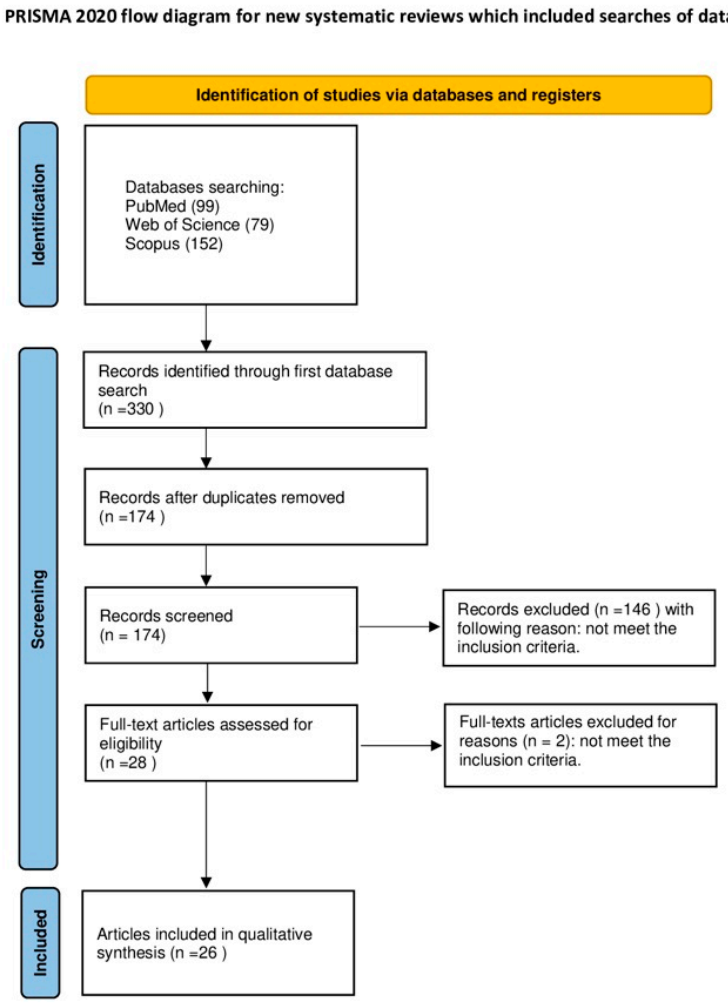

Figure 3. The PRISMA 2020 flow diagram.

2.3. Eligibility Criteria

All studies included in the systematic review had to meet the following criteria: studies examining the antimicrobial properties of gutta-percha, studies examining the effect of adding different ingredients to conventional gutta-percha cones, both in vitro and in vivo studies, studies on human specimens, and studies published in English with no restriction regarding the date of publication. The reviewers agreed upon the following exclusion criteria: studies in non-English languages, studies on non-human specimens, clinical reports, opinions, editorial papers, review articles, and studies without a full-text version available.

2.4. Information Sources, Search Strategy, and Study Selection

On October 30, 2023, we conducted electronic searches in PubMed, Web of Science (WoS), and Scopus databases. In PubMed and WoS, the results were refined to titles, authors, and abstracts, while in the Scopus database, the results were narrowed down to

titles, authors, and keywords. The search criteria were based on the keywords ((antibacterial) OR (antimicrobial)) AND ((gutta-percha) OR gutta-percha)). All searches adhered to the established eligibility criteria, and only articles with available full-text versions were considered.

2.5. Data Collection and Data Items

Four reviewers (J.R, J.K., K.H., W.D.) carefully selected the articles that fulfilled the previously established criteria. The essential data were then collected in a standardized Excel file.

2.6. Assessing Risk of Bias in Individual Studies

During the initial stage of study selection, the authors independently reviewed the titles and abstracts of each study to minimize potential reviewer bias. The level of agreement among reviewers was assessed using Cohen's κ test [28]. Any discrepancies regarding the inclusion or exclusion of a study were resolved through discussions between the authors.

2.7. Quality Assessment

Two independent assessors (J.R, J.K.) conducted an evaluation of the procedural quality for each study included in the article. The assessment criteria were centered around key information pertaining to the association of the antibacterial activity of endodontic gutta-percha. Criteria used to evaluate study design, implementation, and analysis included a minimum group size of 10 subjects, the presence of a control group, a clear description of the performed procedure technique and manufacturer's data, bacterial test quality, and the conduct of tests on teeth. Studies were scored on a scale of 0 to 6 points, where a higher score indicated better study quality. The risk of bias was categorized as follows: 0–2 points denoted a high risk, 3–4 points denoted a moderate risk, and 5–6 points indicated a low risk. Any discrepancies in scoring were resolved through discussion until a consensus was reached.

3. Results

3.1. Study Selection

A comprehensive assessment was initiated with 330 studies, from which 174 duplicates were systematically removed. The scrutiny of titles and abstracts led to the exclusion of 146 articles due to misalignment with the specified subjects or objectives. Further refinement during the full-text examination resulted in the exclusion of an additional two studies that failed to meet the predefined inclusion criteria. The final pool for qualitative synthesis comprised 26 studies (refer to Tables 1–3), composed in English and centered on the in vitro assessment of gutta-percha antibacterial activity, conducted either on human teeth or in laboratory conditions.

3.2. General Characteristics of the Included Studies

The studies incorporated into this systematic review exhibited heterogeneity regarding the specific type of gutta-percha material tested and the microorganisms assessed. The objectives of the included studies focused on the antimicrobial assessment of gutta-percha. Given that the alkalizing potential of the material used for canal obturation exerts a direct influence on microbiota inactivation and tissue healing, this paper measured pH changes after root canal filling with different materials, satisfying the requirements of this systematic review. Most researchers directed their antibacterial evaluations towards *Enterococcus faecalis* [1–5,7–12,15–18,20–22,25,27,29], as it remains the most frequently isolated bacteria from a root canal [30]. Nevertheless, other bacteria underwent analysis, including *Staphylococcus aureus* [4,5,8,13,14,22,23,25,26], *Escherichia coli* [5,13,22,23,26], *Pseudomonas aeruginosa* [4,5,13,14,22,26], *Streptococcus mutans* [4,23,25], *Fusobacterium nucleatum* [18,22,25], *Actinomyces naeslundii* [4,18], *Porphyromonas gingivalis* [4,25], as well as *Peptostreptococcus micros* [16], *Prevotella intermedia* [25], *Porphyromonas endodontalis* [14],

Streptococcus pyogenes [23], *Streptococcus gordonii* [4], *Streptococcus sanguis* [26], *Streptococcus intermedius* [16], *Actinomyces israelii* [18], and *Actinomyces odontolyticus* [22]. Additionally, antimicrobial activity was assessed against fungi, particularly *Candida albicans* [3–5,14,25]. However, Öztan et al. conducted examinations targeting various *Candida* species, including *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, and *Candida tropicalis* [21]. Barthel et al. [6] did not specify the bacteria tested in the study.

The direct contact between bacteria and the filling material was evaluated in two ways—either inside the root canal system of extracted teeth [1,2,6,7,15,17,29] or under laboratory conditions on inoculated plates and Petri dishes [3–5,8–14,16,18,20–23,25,26], specifically fabricated material disks [9], and glass capillaries simulating root canals [27]. It is important to note that, in all the single-canalled teeth used for the experiments (except Lui et al. [7], who did not specify the number of canals in the root system per tooth), Enterococcus faecalis was the only microorganism examined. In compliance with the authors' expectations, most of the articles compared traditional gutta-percha with other root canal fillers or materials based on gutta-percha supplemented with an additional substance/substances. Tennert et al., instead of comparing obturation materials with each other, compared the antibacterial effectiveness of different gutta-percha root canal filling techniques [17]. Additionally, the study by Cook et al. also did not compare the antimicrobial properties of certain final obturation materials with each other; instead, it measured the impact of a temporary chlorhexidine or calcium hydroxide filling on the final gutta-percha antibacterial properties [29].

In the majority of cases, substances incorporated into gutta-percha included chlorhexidine [3,6,7,13,16,21,25–27] and calcium hydroxide [3,6,13,16,21,25–27], with a consistent simultaneous comparison, except in the studies by Lui et al. [7], where calcium hydroxide was utilized as an independent material in paste form, and Ashu Jhamb et al. [13], where calcium hydroxide was both blended with gutta-percha and applied separately as a paste. All cones were procured in prefabricated form across all instances. Other substances combined with gutta-percha comprised iodoform [5,18,22] in the form of MGP, tetracycline in the form of TGP [14,18], and zinc oxide [8,11,16]. When zinc oxide was introduced to gutta-percha, it underwent additional treatment with various materials, such as amoxicillin, clavulanate, ofloxacin–ornidazole [11], chlorhexidine, iodine-polyvinylpyrrolidone [16], or external energy in the form of UV light or Ar Plasma [8].

In comparing obturators and sealers, commonly used for permanent root canal filling alongside gutta-percha, researchers have investigated Resilon [1,10,15,18], AH Plus Jet (AH) [10,12], silver cones [23], BioRott RCS, cobalt–chromium alloy [9], MTA Fillapex, CRCS [12], Apexit Plus, Endomethasone N, Epiphany, and Tubliseal [10]. Vijay Kumar Shakya et al. [12] references a material named gutta-flow in their study, categorized as a sealer but also indicated for final canal obturation according to the producer's specifications. Pawińska et al. [10] also incorporate gutta-flow in their examination, wherein gutta-percha in the form of regular cones is also a focal point. AH Plus remains the primary sealer applied concomitantly with gutta-percha before assessing antibacterial properties [1,2,15,29] (Table 1).

Table 1. General characteristics of included studies.

Authors	Aim of the Study	Material and Methods	Results	Conclusions
Bolhari et al. [1]	Study of antimicrobial activity of MTAD in Human Dentin after obturation of root canals with different obturation materials.	A total of 120 human single-canalled anterior teeth. A total of 90 teeth divided into 3 groups according to their obturation materials; 30 teeth as negative control had no final irrigation with MTAD. Dentin powder exposed to *E. faecalis* for 24 h.	Residual antimicrobial activity of MTAD in the teeth with gutta-percha/AH26 higher than with Resilon/RealSeal SE. Time-dependent decrease in MTAD antimicrobial activity for all groups. The highest antimicrobial activity of MTAD found in the 1-week gutta-percha/AH26 specimens. The lowest antimicrobial activity of MTAD was found in 6-week Resilon/RealSeal SE samples.	Although showing a time-dependent decrease, MTAD maintained its antimicrobial activity at week six. At all-time intervals, Resilon/Epiphany SE significantly reduced the residual antimicrobial activity of MTAD.

Table 1. *Cont.*

Authors	Aim of the Study	Material and Methods	Results	Conclusions
Shantiaee et al. [2]	Study of differences between microleakage in root canals obturated with nanosized silver-coated gutta-percha and standard gutta-percha.	A total of 58 extracted single-rooted teeth. One experimental group obturated with standard gutta-percha and another with nanosized silver-coated gutta-percha. AH26 sealer used as the sealer in both experimental groups. Bacterial leakage investigated after 60 days using *E. faecalis* microbial strains. Dye leakage assessed for 72 h using 1% methylene blue.	Bacterial leakage in 84% of the standard gutta-percha group and 76% of the nanosized silver gutta-percha group. Complete leakage of the dye in 24% and 27% of the standard and nanosized silver gutta-percha groups, respectively. Along the root-end fillings, no significant correlation between dye penetration and bacterial penetration.	Nanosized silver gutta-percha leakage results were comparable to those of standard gutta-percha. Nanosized silver-coated gutta-percha may be more effective in endodontic treatment (antibacterial effects)
Long et al. [9]	Investigating the antimicrobial and ultrastructural properties of root canal filling materials in the presence of biofilm.	Gutta-percha, three root canal sealers, and materials used to make posts. Assessed antimicrobial activity against *E. faecalis*.	Materials' antimicrobial activity varied. The metal alloy posts and BioRoot RCS sealer did not allow biofilm accumulation. Gutta-percha, pulp canal sealer, and resin from fiber-reinforced posts promoted thick biofilm accumulation. In contact with biofilm, microstructural changes were observed in BioRoot and AH Plus.	The impact of the microbial challenge on certain materials resulted in changes to their microstructure, facilitating biofilm accumulation.
Jain et al. [11]	Assessing the antimicrobial efficacy of antibiotic-coated gutta-percha cones on *E. faecalis*.	Gutta-percha cones coated with various medications. Agar plates inoculated with *E. faecalis*. Antibiotic-coated gutta-percha cones were placed on the plates alongside conventional gutta-percha cones, which were coated only with ZOE. To assess the antimicrobial activity, the diameter of the zone of inhibition around the gutta-percha stick was measured.	The discrepancy in the size of the inhibited zone diameter (in mm) for various antibiotics exhibited statistically significant results.	The combination of ZOE and amoxicillin-clavulanic acid proved most effective against *E. faecalis* when compared to other combinations.
Gupta et al. [3]	Evaluation of the effectiveness of chlorhexidine and calcium hydroxide gutta-percha as inter-appointment intracanal medicine against *C. albicans* and *E. faecalis* in vitro.	A total of 3 different sets (their antimicrobial and antifungal capacity tested). The study aims to determine how well chlorhexidine gutta-percha and calcium hydroxide gutta-percha points are effective against *E. faecalis* and *C. albicans*.	A notable variation in the inhibition of *E. faecalis* and *C. albicans* in various materials. Calcium hydroxide GP demonstrated no antimicrobial effect on any of the tested microorganisms.	Chlorhexidine gutta-percha exhibited the highest effectiveness on the tested microorganisms, with maximum efficacy on *E. faecalis*, followed by *C. albicans*. Regular gutta-percha also demonstrated efficiency against the tested microorganisms. Calcium hydroxide gutta-percha had no effect on any of the tested microorganisms.
Moorer et al. [23]	The study aimed to find out whether cleaning gutta-percha cones before putting them in root canals cleaned with sodium hypochlorite is necessary.	Gutta-percha cones contaminated with bacteria and analyzed for the number of bacteria that remained after a certain time span of storage.	Standard endodontic gutta-percha cones inhibited several species of bacteria. Silver points were less effective against *S. aureus* when compared to gutta-percha cones. Bacterial growth in serum can be eliminated with gutta-percha.	Gutta-percha cones possess mild yet noticeable natural antimicrobial abilities that work gradually.
Bozza et al. [25]	Evaluating the antimicrobial activity of gutta-percha points containing antiseptics (calcium hydroxide and chlorhexidine) used for temporary obturation in endodontics.	Gutta-percha points containing calcium hydroxide and chlorhexidine. Gutta-percha points served as control. The zones of bacterial inhibition around each point were measured and the pH values of the broths were recorded.	Gutta-percha points containing chlorhexidine showed inhibition zones with every microorganism. $Ca(OH)_2$ containing points did not inhibit any of the microorganisms. Broth pH values did not exhibit any changes.	The points containing chlorhexidine are more effective antimicrobial agents for the microorganisms tested than the points that contain calcium hydroxide.
Shakya et al. [12]	To investigate the in vitro antimicrobial activity and flow characteristics of resin, calcium hydroxide, mineral trioxide aggregate, and flowable gutta-percha endodontic sealers on *E. faecalis*.	The antibacterial properties of various sealers examined using *E. faecalis* [agar diffusion test (ADT) and direct contact test (DCT)]. The plates spread with standard *E. faecalis*, and the dimensions of inhibition area were measured after 24 h and 7 days. The sealers: AH-Plus, CRCS, MTA Fillapex, and gutta-flow 2	The region of inhibited microbe growth decreased in AH Plus, CRCS, and MTA Fillapex after seven days. DCT exhibited fewer organisms in AH Plus, CRCS, and MTA when compared to controls during both time periods. Gutta-flow 2 did not demonstrate any antimicrobial activity.	The highest level of microbe inhibition observed in CRCS, followed by MTA Fillapex and AH Plus in descending order. Gutta-flow 2 demonstrated no inhibition of *E. faecalis* when combined with ADT. Over time, AH Plus exhibited the most significant decrease in antibacterial activity against *E. faecalis*. AH Plus had the highest flow, while CRCS exhibited the lowest flow.

Table 1. *Cont.*

Authors	Aim of the Study	Material and Methods	Results	Conclusions
Jhamb et al. [13]	The study investigated the antimicrobial efficacy of gutta-percha points impregnated with calcium hydroxide-based paste and several other materials.	Gutta-percha points incorporating $Ca(OH)_2$ and chlorhexidine (Chx), conventional gutta-percha points, and $Ca(OH)_2$ pastes. The antimicrobial effectiveness of Chx and $Ca(OH)_2$ paste compared with $Ca(OH)_2$ points. Antibacterial tests conducted on *P. aeruginosa*, *E. coli*, and *S. aureus*, utilizing the agar disk diffusion method.	All types of microorganisms tested were stopped by gutta-percha dots that had Chx and $Ca(OH)_2$ pastes. No antimicrobial properties were seen with $Ca(OH)_2$ dots or regular gutta-percha dots. The gutta-percha dots containing Chx had the most potent antimicrobial activity.	Gutta-percha points containing chlorhexidine and calcium hydroxide paste exhibited in vitro antibacterial activity against all strains. Calcium hydroxide points, akin to conventional gutta-percha points, lacked antimicrobial activity. Chx points can be considered a more efficient intrathecal drug given the greater time savings and improved ion release profiles.
Tomino et al. [4]	Various concentrations of cetylpyridinium chloride were added to gutta-percha and the antimicrobial properties of these materials were tested.	Thermoplastic gutta-percha supplemented with cationic surfactant cetylpyridinium chloride (CPC) at concentrations of 0.05%, 0.2%, or 0.8%. The gutta-percha-containing CPC tightly packed at the bottom of a 24-well plate. Evaluation of its antimicrobial activity against 8 representative endodontic pathogens (G+,G-,Fungi). Adding 0.5 millilitres of liquid samples containing pathogens to the wells.	Gutta-percha by itself partially inhibited the growth of microorganisms (presence of ZnO). CPC increased the antimicrobial effectiveness of gutta-percha in a dose-dependent way. The addition of 0.05%, 0.2%, and 0.8% CPC caused a reduction in viable microbial number. The lower limit of detection was reached for all tested pathogens, except for *P. aeruginosa*, which was detected in gutta-percha containing 0.8% CPC.	Incorporation of CPC markedly boosted the antimicrobial effectiveness of gutta-percha. Utilization of CPC-infused gutta-percha can help decrease the likelihood of re-infection of the root canal during root canal therapy.
Shur et al. [22]	To investigate whether 'MGP' gutta-percha, a commercially available product that contains iodoform, can inhibit the development of potential endodontic pathogens.	Inocula of *E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. sanguis*, *F. nucleatum* and *A. odontolitycus* spread onto the surface of agar plates. 'MGP' gutta-percha cones presoaked in sterile water were transferred to the inoculated agar and incubated. Identical studies performed using iodoform-free gutta-percha and sterile paper saturated with 10% poviodone-iodine.	Povidone-iodine stopped all types of germs. Iodoform-free gutta-percha stopped *S. sanguis* and *A. odontolyticus*. 'MGP' gutta-percha stopped *S. aureus*, *S. sanguism A. odontolyticus*, and *F. nucleatum*. Neither iodoform-free gutta-percha nor 'MGP' gutta-percha could prevent the growth of *E. faecalis*, *E. coli* or *P. aeruginosa*.	Compared to gutta-percha without iodoform, incorporating iodoform in 'MGP' gutta-percha displayed an inhibitory effect on *S. aureus* and *F. nucleatum* in vitro, while demonstrating no effect on *E. faecalis*, *E. coli*, or *P. aeruginosa*.
Bodrumlu et al. [5]	The objective of this investigation was to assess and juxtapose the antimicrobial and antifungal efficacy of MGP and standard gutta-percha cones at varying time intervals via the disk diffusion technique.	*E. faecalis*, *P. aeruginosa*, *S. aureus*, and *E. coli* applied to Mueller–Hinton agar, while *C. albicans* was applied to Sabouraud agar with added glucose. Subsequently, same-size MGP cones, conventional gutta-percha cones, and povidone–iodine-impregnated disks placed on the inoculated plates.	The povidone-iodine-impregnated disks exhibited bacteriostatic efficacy against all bacterial strains for up to 72 h. Regular gutta-percha cones did not show any inhibition zones. The antimicrobial impact of MGP cones against *E. coli* and *P. aeruginosa* disappeared by 48 and 72 h, respectively. MGP proved more effective than regular gutta-percha. Both MGP and povidone-iodine were effective against *C. albicans* for up to 72 h, while regular gutta-percha did not exhibit any antifungal activity.	The potential antimicrobial and antifungal properties of MGP may provide supplementary benefits compared to traditional gutta-percha.
Emre Bodrumlu et al. [14]	The aim of this study was to assess the antimicrobial and antifungal effectiveness of commercially accessible gutta-percha infused with tetracycline on prospective endodontic pathogens by conducting in vitro analysis.	The bacterial strains used in the experiment included *E. faecalis*, *P. aeruginosa*, *S. aureus*, *P. endodontalis*, and *C. albicans*. The same size tetracycline-integrated gutta-percha (TGP) cones, a tetracycline disk, and conventional gutta-percha cones were placed on agar plates that were inoculated with the bacterial strains.	Tetracycline disks and TGP cones inhibited all the bacterial strains that were tested. The treatments appeared to be less effective on *E. faecalis* and *P. aeruginosa*. None of the tested treatments able to affect *C. albicans*.	TGP appears to provide an antimicrobial benefit compared to traditional gutta-percha.
Tanomaru et al. [26]	The objective of this study was to assess the antimicrobial effectiveness of various brands and formulations of gutta-percha points and calcium hydroxide pastes employed in endodontic treatments.	The studied items were gutta-percha points with calcium hydroxide, gutta-percha points with chlorhexidine, 2 traditional gutta-percha points, and 2 calcium hydroxide pastes. Antibacterial tests conducted with *E. coli*, *S. epidermidis*, *S. aureus*, *P. aeruginosa*, and *M. luteus*. The agar diffusion technique was used.	Inhibition of all microbial species examined by the gutta-percha points containing chlorhexidine and the calcium hydroxide pastes, with similar outcomes. No evidence of antimicrobial activity was observed within the other groups.	Gutta-percha points with chlorhexidine showed antimicrobial properties, but gutta-percha points with calcium hydroxide did not.

Table 1. *Cont.*

Authors	Aim of the Study	Material and Methods	Results	Conclusions
Barthel et al. [6]	The objective of this investigation was to evaluate the antibacterial efficiency of chlorhexidine or calcium hydroxide, integrated with gutta-percha points, when compared to the delivery of calcium hydroxide or chlorhexidine as gel or paste.	A total of 70 roots exposed for a week in the oral cavities of 2 volunteers. The roots were then removed (microbial analysis conducted). Subsequently, the roots were treated with either calcium hydroxide paste, 5% chlorhexidine gel, or a combination of chx and $Ca(OH)_2$ containing gutta-percha points. Subsequently, a thioglycolate-soaked paper point introduced into each canal, and the roots were incubated for an additional week to observe any bacterial regrowth.	After one week of using various medications, the number of bacteria decreased significantly in all test groups when compared to the control group. The CHX-gel and $Ca(OH)_2$-paste groups showed much better results than the CHX-GP or the $Ca(OH)_2$-GP group.	Only the groups treated with chlorhexidine gel and calcium hydroxide paste demonstrated a significant absence of microbial colonization in a sizeable number of samples following 1 and 2 weeks.
Lui et al. [7]	In vitro assessment of the antimicrobial properties of chlorhexidine-impregnated gutta-percha points.	Maxillary premolar teeth removed for orthodontic reasons. The teeth then separated into buccal and palatal roots and adjusted to a length of 10 mm. The root canals prepared to ISO 40 with ProFile files, and the teeth placed in an ultrasonic bath with 17% EDTA for 4 min followed by 5.25% sodium hypochlorite for 4 min to remove any residue. *E. faecalis* grown in BHI broth. A total of 3 treatment groups were prepared, each of 18 roots: Group A—creamy paste made of 10 mg calcium hydroxide powder and 10 mL distilled water, Group B—chlorhexidine-infused gutta percha points of size ISO 40 and length 9 mm, Group C (positive control group)—5 mL saline rinsed into their root canals.	At the depth of 100 mm and 250 mm into the dentine, fewer *E. faecalis* colonies were found in Group A and Group B as compared to the control group. A significant difference observed between the two groups at the 100 mm depth, where chlorhexidine-impregnated gutta-percha points were less effective in reducing *E. faecalis* colonies than calcium hydroxide.	Chlorhexidine-infused gutta-percha points do not sufficiently inhibit the growth of *E. faecalis* in infected dentinal tubules and are also less effective than calcium hydroxide paste.
Panwar et al. [20]	Nanosized curcumin antibacterial potency against *E. faecalis* evaluated in vitro.	Sterilized gutta-percha cones covered with artificial curcumin nanosized particles. A total of 44 agar plates set up to culture *E. faecalis*; 22 plates held normal gutta-percha cones, while the remaining 22 held gutta percha-cones coated with nanosized curcumin.	The minimum inhibitory concentration (MIC) of nanosized curcumin against *E. faecalis* was 50 mg/mL. Nanosized curcumin-coated gutta-percha exhibited a bigger inhibition zone compared to conventional gutta percha (>Inhibitory activity against *E. faecalis*).	The growth of *E. faecalis* can be stopped by nanosized curcumin.
Alves et al. [8]	To enhance the antibacterial efficacy of gutta-percha cones, their surface properties can be modified by argon plasma treatment and subsequent deposition of a thin ZnO layer.	Gutta-percha cones ISO 80 (6 groups): 1. GP cones without modification (control group); 2. GP cones with 5.25% NaOCl ("NaOCl" group); 3. GT cones were subjected to plasma treatment in the argon atmosphere. ("PT group"); 4. Thin film of ZnO was deposited on plasma-treated GP cones by using a Physical Vapor Deposition (PVD) technique ("PT+ZnO" group); 5. Thin film of ZnO was deposited on non-plasma-treated GP cones ("ZnO" group); 6. Activation of deposited ZnO films by UV light ("PT+ZnO+UV" group). All GP cones were incubated with *E. faecalis* and *S. aureus*.	Both GP cones with NaOCl and GP cones with ZnO film resulted in 30% decrease in *E. faecalis* (comparing the control group). GP cones with ZnO film and (PT+ZnO) displayed a significantly enhanced antibacterial effect of a 51% reduction (compared to the control group). UV light activation of the PT+ZnO cones did not result in any extra antimicrobial effect.	The ZnO covering on gutta-percha cones boosted their ability to fight *E. faecalis* and *S. aureus* bacteria. When combined with argon plasma treatment, the ZnO coating showed even stronger antimicrobial effects against *E. faecalis* and *S. aureus*.
Ebert et al. [27]	Assessing the ability of modified gutta-percha cones to kill *E. faecalis* bacteria in simulated root canals.	*E. faecalis* added to a sterile test tube of Schaedler broth. Ten sterile simulated root canals also incubated, each filled with 2.5 µL of bacterial suspension. Two simulated root canals per group filled with various gutta-percha points (all of size ISO 90 and taper 0.02): CHP, calcium hydroxide plus points (CH+P), active points containing chlorhexidine diacetate (AP), conventional gutta-percha points containing zinc oxide (CP), without any gutta-percha points (NP, control group). For assessment of antimicrobial activity of different gutta-percha cones, a colony forming unit (CFU) measured (viable colonogenic cell numbers in CFU/mL).	The control sample without any gutta-percha cones had almost all the bacteria retrieved (99.5%). When gutta-percha cones were present, the recovery level ranged from 0.2% for the AP to 86.9% for the CP. After 5 h of bacterial suspension contact and AP, all bacteria were eliminated. After 10 min, about half of the bacteria were killed in the CHP and CH+P groups, but after 5 h, there was bacterial growth. When compared to NP, CHP did not show a significant difference, but for CH+P, the growth of bacteria was much lower. CP caused growth of *E. faecalis* to increase by 177% when compared to NP.	Both varieties of calcium hydroxide points demonstrated a limited antibacterial effect against *E. faecalis*. Active points containing chlorhexidine diacetate eradicated all the bacteria within 5 h. *E. faecalis* growth was stimulated by traditional gutta-percha points containing zinc oxide.

Table 1. *Cont.*

Authors	Aim of the Study	Material and Methods	Results	Conclusions
Öztan et al. [21]	In vitro evaluation of the sensitivity of *E. faecalis* and the most common *Candida* species to various gutta-percha points.	ISO 40 gutta-percha points containing calcium hydroxide, chlorhexidine diacetate, combination of calcium hydroxide and chlorhexidine diacetate. Microorganisms used: *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, *S. cerevisiae* and *E. faecalis*. All *Candida* strains and *S. cerevisiae* cultured on Sabouraud agar. *E. faecalis* was cultured on 5% sheep blood agar. Portions of 100µL of microorganisms suspended in the diluted serum and transferred to Eppendorf tubes.	Calcium hydroxide plus points or active points did not demonstrate significant antimicrobial activity against *E. faecalis*, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, or *C. tropicalis* within a timeframe of 14 days. GP containing calcium hydroxide or chlorhexidine diacetate was effective against *S. cerevisiae*. CHX/Ca Combi points completely eradicated *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, and *S. cerevisiae*, yet both *E. faecalis* and *C. parapsilosis* remained resistant to CHX/Ca Combi points after 14 days.	Gutta-percha points incorporating a mixture of calcium hydroxide and chlorhexidine diacetate exhibit superior efficacy against certain microorganisms (excluding *E. faecalis* and *C. parapsilosis*) in comparison to either calcium hydroxide or chlorhexidine diacetate alone.
Melker et al. [18]	Evaluation of antibacterial potency of 3 commercially available gutta-percha blends—standard gutta-percha, gutta-percha with iodoform (MGP), gutta-percha with tetracycline—against 15 bacterial species of *A. israeli*, *A. naeslundii*, *F. nucleatum* and *E. faecalis*.	Bacteria collected from cultures, including *E. faecalis* from periapical lesions on retreated teeth. A total of 6 agar plates prepared: one with 5 Resilion points, one with 5 standard gutta-percha points, one with 5 MGP points, one with 5 tetracycline gutta-percha points, one with a sterile paper disk containing tetracycline or a tetracycline E-test strip, and one left empty as a control.	The growth of *A. naeslundii* and *F. nucleatum* slightly stopped by standard GP. MGP more effective than standard GP at stopping the growth of *Actinomyces* spp. and *Fusobacterium*. The growth of *E. faecialis* was not stopped by either MGP or standard gutta-percha. GP with tetracycline showed the greatest antibacterial activity; MGP was better at stopping the growth of *Fusobacterium*. The growth of *Actinomyces* was completely stopped by gutta-percha mixed with tetracycline. Against almost all types of *E. facealis*, tetracycline was effective.	Gutta-percha mixed with tetracycline stopped the growth of all four types of bacteria. MGP stopped the growth of *A. Israelii*, *A. naeslundii* and *F. nucleatum*. Regular gutta-percha blocked the growth of *A. israelii* and *A. naeslundii*, but Resilion was not effective in fighting bacteria. Therefore, it is recommended that gutta-percha mixed with tetracycline is a suitable material for filling root canals.
Pawińska et al. [10]	In vitro comparison of the pH levels of 8 different commercially available points and root canal sealers	The tested materials: AH Plus Jet (AH), Apexit Plus (AP), gutta-flow (GF), Endomethasone N (END), Epiphany (EP), gutta-percha (G), Tubliseal (T) and Resilon (R); 0.1 g of each point and sealer material placed inside dialysis tubes, then inserted into plastic vials containing 20 mL of deionized water. pH values measured immediately after immersion and at intervals of 1, 2, 24, 48, 120, and 198 h.	Epiphany showed the highest pH, followed by Apexit Plus and AH Plus Jet. Resilon had lower pH than gutta-flow and gutta-percha initially, but eventually increased. Gutta-percha had lower pH than gutta-flow during the first 2 h but increased significantly after 48 h. Endomethasone and Tubliseal had relatively insignificant pH values throughout the experiment. Only Epiphany and Apexit Plus exhibited a gradual rise in pH value until the end of the experiment.	AH Plus, Epiphany, Apexit Plus elevate the pH level, which helps to heal periapical tissues and deactivate microorganisms in root canals. Gutta-percha, gutta-flow, and Resilon do not raise the pH sufficiently to support these beneficial processes. Combining the root canal sealer with the main obturation material can stimulate its alkalizing potential.
Bolhari et al. [15]	To evaluate the effect of MTAD on the expression of virulence factors in *E. faecalis* before and after obturation using guttapercha/AH Plus or RealSeal SE/Resilon.	A total of 144 non-carious, single-rooted and single-canalled teeth, with anatomy confirmed by RVG, extracted due to periodontal problems. Teeth instrumented to ISO 35. The suspension of *E. Facelis* injected into the root canals and the teeth incubated; 90 teeth irrigated with MTAD as a final irrigant. Among those, 30 filled with gutta-percha/AH Plus, 30 with Resilon/RealSeal SE, and 30 were left unobturated (positive controls). A total of 54 teeth not irrigated with MTAD. Among those, 12 teeth were filled with gutta-percha/AH Plus, 12 with Resilon/RealSeal SE, and 30 were left unobturated to act as negative controls.	MTAD successful at reducing the expression of virulence factors efa and esp at all three time periods and gel at the one-week time point. In comparing the effectiveness of gutta-percha/AH Plus and Resilon/Real Seal SE against the expression of virulence factors, gutta-percha showed better efficacy in all groups except for the fsr virulence factor. Gutta-percha proved significantly more effective than the positive controls in every group except for the virulence factor fsr, for which no significant difference was detected. Gutta-percha enhances the antibacterial properties of MTAD in all other groups.	The antibacterial properties of MTAD are due to its ability to prevent specific *E. facealis* virulence factors. Additionally, its effectiveness depends on the obturation material used. Research has shown that selecting gutta-percha/AH Plus fillers following MTAD irrigation results in exceptional antibacterial performance and reliable clinical results.

Table 1. *Cont.*

Authors	Aim of the Study	Material and Methods	Results	Conclusions
Podbielski et al. [16]	Measuring the antimicrobial abilities of guttapercha impregnated with calcium hydroxide, zinc oxide (ZnO), zinc oxide and chlorhexidine, (ZnO/CHX), zinc oxide and iodine-polyvinylpyrrolidone (ZnO/ J-PVP), or a mixture of CHX and J-PVP and ZnO (ZnO/CHX/J-PVP).	Gutta-percha points with the size of ISO 80. Strains of chosen bacteria were cultured—*S. intermedius*, *P. micros*, *P. gingivalis*, and *E. faecalis*. Points transferred to Eppendorf tubes containing bacteria suspension, with some tubes left empty to serve as growth controls. Tubes incubated in 37 degrees Celsius and withdrawn at 1, 2, 3, 4, 7, and 14 days. Afterwards, bathed in ultrasonic pulses and vortexed, put on a solid medium, and incubated for 2 days.	ZnO significantly decreased the bacterial count within 14 days in 3 of the 4 species measured and reduced the *P. gingivalis* to undetectable levels after only 4 days. ZnO mixed with CHX or J-PVP or both, greatly increased the antimicrobial effect of ZnO, completely suppressing *S. intermedius* . Addition of both J-PVP and CHX to ZnO did not turn out to obtain additive effect. *P. micros* was observed to be susceptible to a combination of ZnO and CHX; neither ZnO alone nor ZnO with J-PVP decreased the bacterial count in such a significant way as the combination of ZnO and CHX. Calcium hydroxide was observed to possess the greatest antimicrobial efficiency. It took 1 day to completely inhibit *P. gingivalis*.	None of the examined substances possess properties to totally inhibit growth of all root canal pathogens. GP with calcium hydroxide presented the best results against 3 of 4 chosen species of microorganisms.
Tennert et al. [17]	Comparison of different root filling techniques using gutta-percha and epoxy-resin sealer and their impact on antibacterial effect in extracted human teeth.	A total of 108 single-rooted permanent mandibular and maxillary teeth. Teeth shaped chemomechanically, with the ISO 25/08 file; 12 teeth containing bacteria (culture on agar plate was performed) were excluded from the study. Thereafter, teeth were infected with *E. faecalis*. The teeth were then divided into 4 groups: the first group of 24 teeth filled by lateral compaction technique with ProTaper F2 gutta-percha as a main filling and AH Plus; the second group of 24 teeth filled by 25 Protaper Thermafil obturator with AH Plus; the third group of 24 teeth filled by vertical compaction technique using the Beefill system; the fourth group of 24 teeth was left unobturated as a control group. Cultured on blood agar.	Removal of the root canal filling material displayed 99.9% reduction of bacterial count. Experimental groups showed significantly higher inhibition of *E. faecalis* growth than the control group. Between the experimental groups, no significant differences in the reduction of viability of *E. faecalis* were found.	All root canal filling techniques using gutta-percha significantly decreased *E. facealis* in the extracted teeth. The most potent method, showing the highest number of specimens to totally reduce the bacterial count, turned out to be the warm root canal filling technique.
Cook et al. [29]	Assessment of the effect of the root canal obturation against *E. faecalis* persistance, with or without prior temporary filling with calcium hydroxide or chlorhexidine 2%.	85 teeth, root canals instrumented to size ISO 40, autoclaved, and irrigated with NaOCl 5.25%, EDTA 17% and sodium thiosulfate. A total of 80 canals inoculated with *E. faecalis* and 5 left empty as a negative control. Teeth incubated at 37 degrees Celsius for 21 days and replenished with bacteria on days 4 and 7. After incubation, teeth were divided into 3 groups of 25 teeth and 5 teeth used as positive controls. The first group obturated by gutta-percha and AH Plus in lateral condensation technique. The second group, prior to obturation with gutta-percha, was filled with calcium hydroxide for a week, then rinsed with citric acid, saline, and dried. The third group filled with 2% CHX before the obturation.	Neither of the plates, including the positive controls, showed any bacteria growth after the incubation. In the group of teeth filled previously with calcium hydroxide, *E. faecalis* was detected in 100% of the specimens (PCT test). Teeth obturated immediately with gutta-percha showed *E. faecelis* in 92% of the cases, and the CHX specimens showed *E. faecalis* in 80% of the cases.	Study supports application of CHX for 10 min prior to obturation with gutta-percha and AH Plus, to limit *E. faecalis* count. The influence of residual bacteria, being detectable only by PCR, to the outcomes of the long-term treatment is still unknown.

MTAD—Mixture of tetracycline isomer, acid, and detergent; RCS- Root canal sealer; ZOE—zinc oxide eugenol; ZnO—zinc oxide; GP—gutta-percha; MGP—medicated gutta-percha; TGP—tetracycline integrated gutta-percha; $Ca(OH)_2$—Calcium hydroxide; MTA—mineral trioxide aggregate; CHX—chlorhexidine; CPC—cetylpyridynium chloride; ISO—International Organization for Standarization; BHI—brain heart infusion; MIC—minimum inhibitory concentration; J-PVP—iodine polivinylpyrrolidon; *Enterococcus faecalis*—*E. faecalis*; *Staphylococcus aureus*—*S. aureus*; *Streptococcus pyogenes*—*S. pyogenes*; *Fusobacterium nucleatum*—*F. nucleatum*; *Escherichia coli*—*E. coli*; *Streptococcus mutans*—*S. mutans*; *Porphyromonas gingivalis*—*P. gingivalis*; *Prevotella intermedia*—*P. intermedia*; *Pseudomonas aeruginosa*—*P. aeruginosa*; *Streptococcus gordonii*—*S. gordonii*; *Actinomyces naeslundii*—*A. naeslundii*; *Porphyromonas gingivalis*—*P. gingivalis*; *Streptococcus sanguis*—*S. sanguis*; *Actinomyces odontolitycus*—*A. odontolitycus*; *Porphy-romonas endodontalis*—*P. endodontalis*; *Micrococcus luteus*—*M. luteus*; *Staphylococcus epidermidis*—*S. epidermidis*; *Saccharomyces cerevisiae*—*S. cerevisiae*; *Candida albicans*—*C. albicans*; *Actinomyces israeli*—*A. israeli*; *Streptococcus intermedius*—*S. intermedius*, *Peptostreptococcus micros*—*P. micros*.

3.3. Main Study Outcomes

The objective of this study was to assess the antimicrobial properties generated by gutta-percha and to determine which substances, when mixed with gutta-percha, enhanced these properties. The findings across studies revealed diverse and sometimes contradictory outcomes. Various techniques were employed to measure the impact of gutta-percha on bacterial strains. Most studies did not explicitly state whether gutta-percha alone functioned as an inhibitor of bacterial development. This ambiguity arose from the common practice of comparing regular, classic gutta-percha cones with those enriched with additional substances. These studies focused on describing the improved properties of gutta-percha, while often overlooking a crucial piece of information—the antimicrobial properties of clean gutta-percha.

Nevertheless, six papers [1,3,15,18,20,23] provided a clear answer, affirming that clean gutta-percha, without any additional substances, did indeed exhibit antimicrobial properties. Among these, five studies [1,3,15,20,23] demonstrated antibacterial efficacy specifically against *Enterococcus faecalis*. It was established that gutta-percha inhibited every *Enterococcal* virulence factor except the fsr virulence factor, which did not exhibit significant differences upon contact with the material [15]. Additionally, gutta-percha was found to inhibit the growth of *Candida albicans*. Furthermore, Melker et al. [18] reported that gutta-percha alone actively combated the species of *Actinomyces israelii* and *Actinomyces naeslundii*. Moorer et al. [23] suggested that gutta-percha inhibited several endodontic species; however, the study did not specify which ones.

However, six studies asserted that gutta-percha lacked antibacterial and antifungal properties [5,9,10,12,14,26] against the microbial species used in the experiments. Ebert et al. [27] extended these conclusions further, indicating that, in a study using glass capillaries to simulate root canals, gutta-percha containing zinc oxide, when used to fill the capillaries, led to a 177% increase in the growth of *Enterococcus faecalis* compared to the empty infected samples. Interestingly, different results emerged in studies comparing gutta-percha and Resilon. Some studies [1,15,18] asserted that gutta-percha exhibited superior antibacterial properties compared to Resilon. However, a study by Pawińska et al. [10] demonstrated that both gutta-percha and Resilon did not exhibit any antimicrobial effect.

The results from five studies [3,13,25–27] consistently demonstrated the superiority of chlorhexidine over the addition of calcium hydroxide to gutta-percha. In a singular study [16], contrasting findings were reported. The efficacy of chlorhexidine against *Enterococcus faecalis* was consistently confirmed in four studies [3,13,25,27]. The addition of calcium hydroxide to gutta-percha exhibited activity against this specific strain of bacteria in two studies [16,27]. Notably, in one study, calcium hydroxide paste exhibited superior antibacterial properties compared to gutta-percha with chlorhexidine [7]. Barthel et al. [6] investigated chlorhexidine and calcium hydroxide individually, determining that each possessed better antibacterial properties alone than when mixed with gutta-percha. Öztan et al. [21] presented a case in which a combination of both chlorhexidine and calcium hydroxide with gutta-percha demonstrated better performance than a mix of each of these materials with gutta-percha. Unfortunately, none of the examined combinations in the study were effective against *Enterococcus faecalis*.

The addition of iodoform to gutta-percha consistently resulted in an enhancement of the material's properties [5,18,22]. However, Shur et al. [22] asserted that medicated gutta-percha (MGP) remains inactive against *E. faecalis*, *E. aeruginosa*, and *E. coli*. Tetracycline gutta-percha cones (TGP) [14,18] also improved the cones' antibacterial properties and demonstrated effectiveness against *E. faecalis*. Unfortunately, no activity against *Candida albicans* was observed in this case [14]. The supplementation of zinc oxide was found to enhance the examined properties of gutta-percha, and argon plasma treatment further ameliorated these properties. Other substances, namely amoxicillin, clavulanate, ofloxacin–ornidazole [11], cetylpyridinium chloride-CPC [4], and nanosized curcumin [20], were also observed to refine gutta-percha's antibacterial properties. Shantiaee et al. reported that the addition of nanosized silver particles to gutta-percha did not influence the

leakage degree, but the examined cones exhibited beneficial antibacterial properties [2] (Table 2).

Table 2. Detailed characteristics of included studies.

Authors	Microorganism	Medium of Contact: Obturation Material and Bacteria Culture	Materials Examined and Method of Root Canal Obturation	Material Added to Gutta Percha, Its Concentration, and Way of Fusion	Impact of Added Material	Temporary Root Canal Filling	Results (Antimicrobial Properties)
Bolhari et al. [1]	*E. faecalis*	120 single-canalled anterior teeth.	Gutta-percha/AH Plus lateral compaction technique Resilon/RealSeal SE lateral compaction technique	none	n/a	none	Antimicrobial activity of gutta-percha/AH Plus obturaion higher than Resilon/RealSeal SE
Shantiaee et al. [2]	*E. faecalis*	58 single-rooted anterior teeth.	Gutta-percha/AH plus lateral condensation technique Gutta percha with nanosilver/AH Plus lateral condensation technique	Gutta-percha coated with nanosilver particles	No impact on leakage of obturation	none	Better antimicrobial effects of nanosized silver gutta-percha, not measured by the authors
Long et al. [9]	*E. faecalis*	9–11 mm in diameter, 2 mm high disks made of materials.	Gutta-percha, pulp canal sealer, zinc oxide eugenol-based sealer, BioRott RCS, cobalt–chromium alloy	none	n/a	none	All the materials developed biofilm formation, except the metal alloy and BioRoot RCS
Jain et al. [11]	*E. faecalis*	Examination of agar plates.	Gutta-percha coated with ZOE, gutta-percha coated with ZOE + Amoxicillin, guttapercha coated with ZOE + amoxicillin + clavulanate, guttapercha coated with ZOE + ofloxacin-ornidazole.	Single dose of antibiotic added to ZOE cement. Cones coated using varnish.	All antibiotics improved antibacterial properties. Most effective for amoxicillin + clavulanate, then amoxicillin alone, then ofloxacin–ornidazole	none	All added materials presented antimicrobial properties. Antibiotics, especially amoxicillin with clavulanate, significantly increased the antibacterial action.
Gupta et al. [3]	*E. faecalis*, *C. albicans*	Examination of Petri plates.	Gutta-percha, CHX-GP (active points by Reoko Company Germany), calcium hydroxide GP (calcium hydroxide plus points by Roeko, Germany).	Chlorhexidine and calcium hydroxide integrated in the cones.	Adding chlorhexidine to gutta-percha increased its antimicrobial properties, Adding calcium hydroxide to gutta-percha decreased its antimicrobial properties	none	CHX-GP showed the best antimicrobial effect, followed by regular gutta-percha. Calcium hydroxide guttapercha did not show any antimicrobial effect.
Moorer et al. [23]	*S. aureus*, *S. pyogenes*, *E. coli*, *S. mutans*	Examination on agar plates.	Gutta-percha, silver cones.	none	n/a	none	Gutta-percha showed better antimicrobial activity than silver cones against *S. aureus*; however the effect was relatively weak. Authors claim that several endodontic bacterial species could be inhibited by GP only.
Bozza et al. [25]	*S. mutans*, *E. faecalis*, *S. aureus*, *C. albicans*, *F. nucleatum*, *P. gingivalis*, *P. intermedia*	Examination on agar plates.	Gutta-percha, guttapercha with CHX (Roeko), gutta-percha with calcium hydroxide (Roeko), gutta-percha with calcium hydroxide (hygienic).	Calcium hydroxide and chlorhexidine integrated in the cones.	CHX improved the antimicrobial activity of gutta-percha.	none	Points containing CHX show better antimicrobial action than traditional GP and gutta-percha with calcium hydroxide, regardless of the producer.
Shakya et al. [12]	*E. faecalis*	Examination of agar plates.	Gutta-flow 2, AH Plus, MTA Fillapex, CRCS.	none	n/a	none	Gutta-percha material was the only one not to show any antimicrobial action. The highest antibacterial effect was shown by CRCS, followed by MTA and calcium hydroxide.

Table 2. *Cont.*

Authors	Microorganism	Medium of Contact: Obturation Material and Bacteria Culture	Materials Examined and Method of Root Canal Obturation	Material Added to Gutta Percha, Its Concentration, and Way of Fusion	Impact of Added Material	Temporary Root Canal Filling	Results (Antimicrobial Properties)
Jhamb et al. [13]	*P. aerginosa*, *E. coli*, *S. aureus*.	Examination on Petri dishes.	Gutta-percha, gutta-percha with calcium hydroxide, gutta-percha with clorhexidine, calcium hydroxide pastes.	Calcium hydroxide, chlorhexidine integrated in the cones.	CHX added to GP, inhibited the bacterial count.	none	Both chlorhexidine gutta-percha cones and calcium hydroxide pastes showed antibacterial activity against all examined strains. Gutta-percha and gutta-percha + calcium hydroxide cones did not show antimicrobial activity.
Tomino et al. [4]	*E. faecalis*, *S. aureus*, *S. gordonii*, *S. mutans*, *A. naeslundii*, *P. aeruginosa*, *P. gingivalis*, *C. albicans*	Examination on agar plate.	Gutta-percha with 0.05%, 0.2%, and 0.4% of cationic surfactant cetylpyridinium chloride (CPC).	CPC-supplemented thermoplastic gutta-percha	The bigger the concentration, the higher the antimicrobial effect.	none	CPC increased the antibacterial effect of gutta-percha. An 0.8% concentration decreased the level of bacteria to undetectable, except *P. aeruginosa*, although its count significantly decreased
Shur et al. [22]	*E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. sanguis*, *F. nucleatum*, *A. odontolitycus*	Examination on agar plates	Gutta-percha, MGP gutta-percha cones (with iodoform).	Iodoform integrated in the cones.	Inhibited the growth of 4 strains, *S. aureus*, *S. sanguis*, *A. odontolitycus*, and *F. nucleatum*, 2 more than regular gutta-percha, which inhibits only 2 strains: *S. sanguis* and *A. odontolitycus*.	none	Addition of iodoform extended the antimicrobial action of gutta-percha; however, it did not obtain total antibacterial action as it was non-effective against *E. faecalis*, *E. coli* and *P. aeruginosa*
Bodrumlu et al. [5]	*E. faecalis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *C. albicans*	Examination on agar plates	Gutta-percha, MGP gutta-percha cones (with iodoform), disks impregnated with povidone-iodine	Iodoform integrated in the cones.	Increased antibacterial activity of gutta-percha against all bacterial strains but for limited period of time—against *E. coli* for 48 h, against *P. aeruginsa* for 72 h.	none	Povidone-iodine disks showed the best antibacterial and antifungal activity, followed by MGP cones showing antimicrobial effect for a limited time; regular guttapercha showed no antibacterial and no antifungal activity
Emre Bodrumlu et al. [14]	*E. faecalis*, *P. aeruginosa*, *S. aureus*, *Porphyromonas endodontalis*, *C. albicans*	Examination on agar plates	Gutta-percha, tetracycline gutta-percha (TGP), tetracycline Bio-disk.	Tetracycline integrated in the, con, es.	Increased antimicrobial activity of gutta-percha; did not increase antifungal activity.	none	Both TGP and Bio-disks inhibited all tested bacterial strains, with strongest action against *S. aureus* and weakest against *E. faecalis* and *P. aeruginosa*. Gutta-percha did not show any antimicrobial effect. Neither of the materials was active against *C. albicans*
Tanomaru et al. [26]	*M. luteus*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*	Examination on agar plates.	Gutta-percha, gutta-percha with calcium hydroxide (RoekoTM), gutta-percha with chlorhexidine (RoekoTM), two, two calcium hydroxide pastes.	Calcium hydroxide and chlorhexidine integrated in the cones.	Chlorhexidine increased antimicrobial activity; calcium hydroxide did not	none	All examined groups of bacteria were inhibited by gutta-percha with chlorhexidine and by calcium hydroxide pastes. Gutta-percha with calcium hydroxide and conventional gutta-percha did not show any antibacterial activity.
Barthel et al. [6]	Not specified	75 single-rooted teeth	5% CHX gel, calcium hydroxide paste, CHX-GP, gutta-percha with calcium hydroxide (hydroxide plus).	Chlorhexidine and calcium hydroxide integrated in the cones.	Decreased the count of pathogens in the samples compared to positive control group.	none	CHX gel and calcium hydroxide alone showed significantly better antimicrobial properties than gutta-percha cones consisting of these materials. CHX-GP performed better than calcium hydroxide-GP.

Table 2. *Cont.*

Authors	Microorganism	Medium of Contact: Obturation Material and Bacteria Culture	Materials Examined and Method of Root Canal Obturation	Material Added to Gutta Percha, Its Concentration, and Way of Fusion	Impact of Added Material	Temporary Root Canal Filling	Results (Antimicrobial Properties)
Lui et al. [7]	*E. faecalis*	27 maxillary premolars.	Gutta-percha with chlorhexidine (active points), calcium hydroxide paste.	Chlorhexidine integrated in the cones.	n/a (comparison with conventional gutta-percha not possible).	none	Chlorhexidine gutta-percha cones did not inhibit *E. faecalis* totally and were less effective than calcium hydroxide paste.
Panwar et al. [20]	*E. faecalis*	Examination on agar plates.	Gutta-percha, nanocurcumin-covered gutta-percha.	Gutta-percha coated with nanocurcumin particles.	Nanocurcumin increased antibacterial properties of gutta-percha.	none	Both gutta-percha and nanocurcumin-coated gutta-percha showed antimicrobial effects.
Alves et al. [8]	*E. faecalis*, *S. aureus*	Direct and indirect assays.	Gutta-percha, guttapercha immersed in NaOCl 5,25%, guttapercha plasma treated, gutta-percha with ZOE, gutta-percha plasma treated with ZOE, gutta-percha plasma treated with ZOE activated with UV light.	ZOE film deposited on the surface	Around 30% reduction of bacterial count. ZOE-activated treated with plasma reduced bacterial count by 50% compared to control group.	none	Coating with ZOE increased material's antibacterial properties. Additional argon plasma treatment yielded even better results.
Ebert et al. [27]	*E. faecalis*	10 glass capillaries simulating root canals.	Gutta-percha, gutta-percha with calcium hydroxide, gutta-percha with chlorhexidine.	Calcium hydroxide, chlorhexidine integrated in the cones.	Calcium hydroxide limited the count of *E. faecalis*. Chlorhexidine successfully killed all bacteria.	none	Gutta-percha calcium hydroxide presented with good antibacterial effect, whereas gutta-percha chlorhexidine points completely removed bacteria. Clean gutta-percha points turned out to stimulate bacterial growth compared to not-obturated canals.
Öztan et al. [21]	*E. faecalis*, *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida krusei*, *Candida tropicalis*, *S. cerevisiae*	Examination of agar plates	Gutta-percha with calcium hydroxide, gutta-percha with chlorhexidine diacetate, gutta-percha with calcium hydroxide and chlorhexidinediacetate	Calcium hydroxide, chlorhexidine integrated in the cones.	Substances alone were successful only against *Saccharomyces cerevisiae*. When put together, they were active against all types of microorganisms except *E. faecalis* and *C. parapsilosis*	none	Addition of both substances turned out to obtain much higher antimicrobial effect then addition of each substance alone; none of the points showed activity against *E. faecalis* or *C. parapsilosis*
Melker et al. [18]	*E. faecalis*, *Actinomyces israeli*, *A. naeslundii*, *F. nucleatum*	Examination of agar plates	Gutta-percha, gutta-percha with iodoform (MGP), gutta-percha with tetracycline, Resilon.	Iodoform, tetracycline integrated in the cones.	Iodoform increased activity against actinomyces spp. And fusobacterium. Tetracycline was active against all types of bacteria.	none	Gutta-percha with tetracycline turned out to have the highest antimicrobial potency, followed by gutta-percha with iodoform, followed by regular gutta-percha (active against *A. israeli* and *A. naeslundii*)
Pawińska et al. [10]	none	Examination of vials.	AH Plus Jet (AH), Apexit Plus (AP), Endomethasone N (END), Epiphany (EP), gutta-flow (GF), gutta-percha (G), Resilon (R) and Tubliseal (T).	none	n/a	none	Gutta-percha, gutta-flow and Resilon did not present any antimicrobial effect. AH Plus, Apexit Plus, and Epiphany did.
Bolhari et al. [15]	*E. faecalis*	144 single-rooted, single-canalled teeth.	Gutta-percha/AH Plus, Resilon/RealSealSE, lateral condensation technique.	none	n/a	none	Gutta-percha provided much better antibacterial effect than Resilon

Table 2. *Cont.*

Authors	Microorganism	Medium of Contact: Obturation Material and Bacteria Culture	Materials Examined and Method of Root Canal Obturation	Material Added to Gutta Percha, Its Concentration, and Way of Fusion	Impact of Added Material	Temporary Root Canal Filling	Results (Antimicrobial Properties)
Podbielski et al. [16]	*S. intermedius*, *P. micros*, *P. gingivalis*, *E. faecalis*	Examination in Eppendorf tubes.	Gutta-percha with calcium hybdroxide, gutta-percha with zinc oxide, gutta-percha with zinc oxide and chlorhexidine, gutta-percha with zinc oxide and iodine-polyvinylpyrrolidone, gutta-percha with a mixture of CHX and J-PVP and ZnO	Calcium hydroxide, zinc oxide, chlorhexidine, iodine-poly-vinylpyrrolidone, and a mix of those; materials integrated in the ones.	n/a (comparison with conventional gutta-percha not possible).	none	Gutta-percha with calcium hydroxide showed the best antibacterial activity against 3 of 4 chosen species.
Tennert et al. [17]	*E. faecalis*	108 single-rooted permanent mandibular and maxillary teeth.	Gutta-percha and epoxy resin sealer.	none	n/a	none	Gutta-percha with epoxy resin sealer used to fill canals with different methods inhibited the bacterial count.
Cook et. al. [29]	*E. faecalis*	85 single-canalled teeth.	Gutta-percha and AH Plus sealer.	none	n/a	Chlorhexi-dine filling for 10 min, calcium hydrox-ide filling for a week.	Temporary filling with CHX significantly decreased *E. faecalis* count.

MTAD—Mixture of tetracycline isomer, acid, and detergent; RCS- Root canal sealer; ZOE—zinc oxide eugenol; ZnO—zinc oxide; GP—gutta-percha; MGP—medicated gutta-percha; TGP—tetracycline integrated gutta-percha; Ca(OH)2—Calcium hydroxide; MTA—mineral trioxide aggregate; CHX—chlorhexidine; CPC—cetylpyridynium chloride; ISO—International Organization for Standarization; BHI—brain heart infusion; MIC—minimum inhibitory concentration; J-PVP—iodine polivinylpyrrolidon *Enterococcus faecalis—E. faecalis; Staphylococcus aureus—S. aureus; Streptococcus pyogenes—S. pyogenes; Fusobacterium nucleatum—F. nucleatum; Escherichia Coli—E. coli; Streptococcus mutans—S. mutans; Porphyromonas gingivalis—P. gingivalis; Prevotella intermedia—P. intermedia; Pseudomonas aeruginosa—P. aeruginosa; Streptococcus gordonii—S. gordonii; Actinomyces naeslundii—A. naeslundii; Porphyromonas gingivalis—P. gingivalis; Streptococcus sanguis—S. sanguis; Actinomyces odontolitycus—A. odontolitycus; Porphyromonas endodontalis—P. endodontalis; Micrococcus luteus—M. luteus; Staphylococcus epidermidis—S. epidermidis; Saccharomyces cerevisiae—S. cerevisiae; Candida albicans—C. albicans; Actinomyces israeli—A. israeli; Streptococcus intermedius—S. intermedius*, *Peptostreptococcus micros—P. micros*.

3.4. Quality Assessment

Out of the articles included in the review, two [15,17] were deemed high-quality with a score of 6/6 points. Fourteen studies [3–5,12–15,20–23,26,27] were classified as low-quality. Additionally, ten studies [1,2,6,7,9–11,18,20,23–25] were considered to have a moderate risk of bias, scoring between 3 and 4 points (Table 3).

Table 3. Assessing risk of bias, presence (1) or its absence (0).

	Group Size at Least 10 Subjects	Control Group	Sample Size Calculation	Detailed Description of Procedure	Bacterial Test Quality: Molecular Test (1) or Culture (0)	Study with (1) or without (0) Teeth	Total Points	Risk of Bias
Bolhari et al. [1]	1	1	0	1	0	1	4	Moderate
Shantiaee et al. [2]	1	1	0	1	0	1	4	Moderate
Long et al. [9]	1	1	0	1	0	0	3	Moderate
Jain et al. [11]	1	1	0	1	0	0	3	Moderate
Gupta et al. [3]	0	1	0	1	0	0	2	High
Moorer et al. [23]	0	1	0	0	0	0	1	High
Bozza et al. [25]	1	1	0	1	0	0	3	Moderate
Shakya et al. [12]	0	1	0	1	0	0	2	High
Jhamb et al. [13]	0	1	0	1	0	0	2	High
Tomino et al. [4]	0	1	0	1	0	0	2	High
Shur et al. [22]	1	1	0	1	0	0	2	High
Bodrumlu et al. [5]	0	1	0	1	0	0	2	High
Emre Bodrumlu et al. [14]	0	1	0	1	0	0	2	High

Table 3. *Cont.*

	Group Size at Least 10 Subjects	Control Group	Sample Size Calculation	Detailed Description of Procedure	Bacterial Test Quality: Molecular Test (1) or Culture (0)	Study with (1) or without (0) Teeth	Total Points	Risk of Bias
Tanomaru et al. [26]	0	1	0	1	0	0	2	High
Barthel et al. [6]	1	1	0	1	0	1	4	Moderate
Lui et al. [7]	0	1	0	1	0	1	3	Moderate
Panwar et al. [20]	1	0	0	0	0	0	1	High
Alves et al. [8]	0	1	0	1	0	0	2	High
Ebert et al. [27]	0	1	0	1	0	0	2	High
Öztan et al. [21]	0	1	0	1	0	0	2	High
Melker et al. [18]	1	1	0	1	0	0	3	Moderate
Pawińska et al. [10]	1	1	0	1	0	0	3	Moderate
Bolhari et al. [15]	1	1	1	1	1	1	6	Low
Podbielski et al. [16]	0	1	0	1	0	0	2	High
Tennert et al. [17]	1	1	0	1	0	1	4	Moderate
Cook et. al. [29]	1	1	1	1	1	1	6	Low

4. Discussion

In contemporary dentistry, a rapidly advancing scientific field, efforts are directed toward enhancing the quality of dental materials and devices to optimize treatment outcomes [31–35]. A multitude of research focuses on dental materials, encompassing those used in endodontics, with the objective of improving their properties. This study aimed to identify potential approaches for increasing the antibacterial activity of gutta-percha by introducing additional substances into its composition. The results obtained indicated that there is no singular ideal component capable of significantly enhancing the antimicrobial and antifungal properties of gutta-percha. None of the materials added to gutta-percha demonstrated a comprehensive improvement in its antimicrobial efficacy, including activity against *Enterococus faecalis*. However, the addition of certain studied substances may indeed enhance the antimicrobial potency of gutta-percha.

According to the studies incorporated in the present review, the most notable quantitative outcomes are observed with the addition of chlorhexidine. Chlorhexidine, a synthetic antiseptic agent, effectively aids in reducing bacterial and fungal counts within the canal system, even against highly resistant strains such as *E. faecalis*. The antimicrobial properties of chlorhexidine are also applicable in a single-substance temporary root canal filling [29]. Based on the analyzed studies, iodoform may be an underappreciated yet promising material to incorporate into gutta-percha. Iodoform exhibits antimicrobial activity through the release of iodine [36], and its addition to gutta-percha resulted in a 100% enhancement of the material's properties in all cases. Unfortunately, only three papers included in this review provided studies on MGP gutta-percha.

The addition of calcium hydroxide to gutta-percha has been shown to enhance antibacterial properties. Calcium hydroxide, considered one of the oldest biomaterials used in endodontics, was introduced by Hermann in 1920 in Germany [37]. The fundamental action of calcium hydroxide involves ionic dissociation into hydroxyl ions and calcium ions, exerting a positive effect on microorganisms and tissue healing processes [38]. Hydroxyl ions of calcium hydroxide act on enzymes in the cell membrane, contributing to its wide-ranging antimicrobial activities, effective against various microorganisms, irrespective of their metabolic capabilities [38]. However, the combination of gutta-percha and calcium hydroxide does not seem to exhibit antimicrobial activity against *E. faecalis*, highlighting a limitation in the material. Further research is warranted on silver [2], cetylpyridinium chloride-CPC [4], and nanocurcumin [20] incorporated into gutta-percha.

Taking all aspects into consideration, the authors demonstrated that gutta-percha alone may exhibit antibacterial activity, including activity against *E. faecalis*. These findings align with Moorer et al. [39], who assert that zinc oxide, the primary component of gutta-percha cones, contributes to these antibacterial properties. Indeed, additional zinc oxide supplementation enhances the antimicrobial action of the cones [7,10,23]. The most significant antimicrobial effects are achieved when using gutta-percha with chlorhexidine, indicating the greatest reduction in bacterial cultures after contact with this substance. Study results

indicated that chlorhexidine is effective against *Enterococcus faecalis*, a notion supported by numerous other studies demonstrating both the in vitro and in vivo effectiveness of this aspect [40–47]. Furthermore, chlorhexidine exhibits biocidal activity against Gram-positive bacteria, and, if the minimum inhibitory concentration (MIC) is maintained, it can also inhibit Gram-negative bacteria. Additionally, chlorhexidine remains active against DNA and RNA viruses and yeast, including *Candida albicans* [48–53].

The primary limitation of this systematic review is that the considered studies were based on both in vitro and in vivo examinations of the samples. Consequently, prior to examination, the materials underwent varied and non-uniform conditions, even within the scope of each group. Materials tested in vivo were intracanal, placed after canal instrumentation and disinfection. The canal system irrigation varied across studies and included the use of MTAD, EDTA 17% or 20%, NaOCl 3% or 5, 25%, 0.9% NaCl, or saline of unknown concentration. The selection of different root canal rinsing agents undoubtedly impacts the post-preparation microflora of the endodontium [54–57] and the quality of obturation. Moreover, not only does the type of irrigant affect overall root canal treatment efficacy, but also its volume and the duration of rinsing. The choice of the preparation technique and the tooth type are also crucial factors.

Another limitation pertained to the duration of bacterial culture incubation on the material's surface in in vitro conditions, ranging from a minimum of 1 h [30] to a maximum of 21 days [20]. Bacterial growth does not follow a consistent vector over time, leading to different periods of incubation yielding diverse, non-uniform, and non-comparable counts of microorganisms. In future studies, it is imperative for researchers to standardize laboratory conditions and root canal system preparation before obturation. Additionally, each study should prioritize assessing material activity specifically against *Enterococcus faecalis*, considering this bacterium as the primary pathogen associated with root canal treatment [58–63].

5. Conclusions

Eradicating bacteria from the root canal lumen and obturating it with a suitable material possessing antimicrobial properties can create conducive conditions for the long-term preservation of the tooth in the oral cavity. Despite its limited efficacy against certain endodontic pathogens, gutta-percha alone is incapable of providing a durable, consistent antibacterial effect in the tissues. Gutta-percha cones incorporating chlorhexidine, iodoform, calcium hydroxide, and zinc oxide warrant further investigation due to the promising results identified in this review. It is crucial to acknowledge that retaining a tooth in the mouth positively influences the patient's motivation for ongoing treatment and the maintenance of proper oral hygiene, leading to more frequent dental check-ups and the preservation of healthy teeth. Additionally, one must consider the occlusal and orthodontic issues a patient may face after tooth loss. Sustaining proper dentition will enhance the patient's comfort and diminish the risk of expensive prosthetic restorations or implants, often indicated in cases of tooth loss.

Author Contributions: All authors contributed equally to the manuscript preparation. All authors have read and approved the final article. Conceptualization, M.D.; methodology, K.H. and J.K.; software, J.K., J.R., K.H. and W.D.; validation, J.K. and J.M.; investigation, J.K., J.R., K.H. and W.D.; data curation, J.K.; writing—original draft preparation, J.K., J.R., K.H., W.D. and J.M.; writing—review and editing, J.M., M.D. and R.J.W.; supervision, J.M., M.D. and R.J.W.; project administration, M.D. and R.J.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financed by a subsidy from Wroclaw Medical University, number SUBZ.B180.23.054.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: Authors Jakub Kowalski, Joanna Rygas, and Karolina Homa were employed by the following companies: Dental Practice, Dental Focus, Babiogórska 34, 43-400 Bielsko-Biała, Poland; Dental Practice, Wojciecha z Brudzewa 10, 51-601 Wroclaw, Poland; and NZOZ Medident, Żeromskiego 2A, 43-230 Goczałkowice-Zdrój, Poland, respectively. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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