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Review Article

The potential of chitosan-silver nanoparticle-graphene oxide hybrid as an antimicrobial therapy against uropathogenic resistance in urinary tract infections

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ABSTRACT

Urinary tract infections (UTIs) are among the most common infections globally, with 150-250 million annual cases. In recent years, there has been an emerging trend of complicated UTIs, partly due to the resistance of uropathogens to commonly used first-line antibiotics. At its worst, antimicrobial resistance increases in-patient time, treatment costs, and the risk of complications and mortality. Resistance also gravely reduces treatment options. The writers review the literature published in 2013-2023 found on Pubmed, EBSCO-Host, and Google Scholar using several combination keywords: "new treatment," "urinary tract infection," "bacteria," "antimicrobial," and "resistance." Thirty-three *in-vitro*, three animal models (*in-vivo*), and one multicentre cohort study were found on the composite chitosan, silver nanoparticle, and graphene oxide (Chit-AgNP-GO), and its components. This bioactive material possesses a bactericidal and bacteriostatic effect against Gram-positive and Gram-negative uropathogens. Chit-AgNP-GO has multiple mechanisms of action for each component involved, with literature suggesting good antimicrobial activity for each. Silver destroys bacterial enzymes necessary for electron transfer, disrupts DNA synthesis, and destroys proteins integral to the bacterial structure. Chitosan facilitates adhesion to bacterial cell membranes and can contribute to bacterial cellular leakage. Graphene oxide affects bacterial liposolubility and permeability. The multiple mechanisms of action generated by this antimicrobial make it a promising agent to study in UTI cases, especially those complicated by uropathogenic resistance. However, validating this nanomaterial may be challenging due to the limited number of clinical studies. Therefore further *in vivo*, and clinical research protocols are highly required to confirm its antimicrobial potential in the clinical setting.

Keywords:
Antimicrobial, Complicated UTI, Chitosan, Silver nanoparticle, Graphene oxide

1. INTRODUCTION

Urinary tract infection (UTI) is an infection marked by the growth of bacteria in the urinary tract starting from 10^4 to 10^6 colony-forming units/millilitre (CFU/mL) of urine[1]. UTI is the most common infection globally, with 150-250 million cases annually. It is estimated that 40-50% of women and 5% of men will suffer from UTI at least once in their lifetime, making UTI the most common infectious disease in the general population even today[2]. The causative pathogens of UTI are several Gram-positive and Gram-negative bacteria[1-2]. Amongst Gram-negative bacteria, the uropathogenic strain of *Escherichia coli* (UPEC) causes 75-90% of all UTIs, while staphylococci are the most common Gram-positive bacteria to cause UTIs, causing 5-10% of all uncomplicated UTI[2-3].

Antibiotic resistance in uropathogens is a worldwide issue, affecting both developed and developing countries. Resistance data in UTIs vary across countries. In Central Europe, antimicrobial resistance rates drive the use of antibiotics for UTIs away from readily available first-line drugs[4], with data from one study of lower UTIs in Milan, Italy confirming increased resistance of *E. coli* isolates against ampicillin (51% susceptible), cotrimoxazole (76%

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susceptible), and amoxicillin-clavulanic acid (77% susceptible)[5]. Developing countries might encounter a higher burden of antimicrobial resistance in uropathogens due to the common misuse of antibiotics. Asia, and particularly Indonesia, is not exempt from this trend. A systematic review on uropathogenic resistance in the Asia-Pacific region reports a high prevalence of resistance against cotrimoxazole, ciprofloxacin, and ceftriaxone. (33-90%). Along with Bangladesh, India, and Sri Lanka, Indonesia has the highest prevalence of resistance[6]. These circumstances show that new antimicrobial compounds are needed to stand against this emerging trend.

Uropathogenic resistance is a challenge to the medical field because it causes extended in-patient time, increased treatment costs, and increased risk of complications and death[7]. Chronically untreated UTIs may lead to complications such as pyelonephritis, scar formation in the urinary tract, and urethral strictures, which are more common in men[8-10]. In pregnant women, UTIs can increase the risks of low birth weight and premature births[8,11]. Resistance limits treatment options, making emerging therapy options essential. One such therapy is the use of bioactive materials such as silver, which is used medically as wound dressings, creams, and an antibiotic coating on medical devices due to its antimicrobial activity[12]. Silver formulated as nanoparticles (AgNP) exerts higher bactericidal action due to their enhanced reactivity resulting from their high surface/volume ratio. The nanoparticle is shown to eliminate Gram-positive bacteria with low minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)[13]. AgNP also shows synergistic effects with other antimicrobial drugs. However, AgNP can be unstable and quickly deposited when transferred to biological media, reducing its potency. Therefore, a supporting matrix that amplifies stability and antibacterial properties is required, provided by graphene oxide (GO), a derivative of graphite. A large surface area makes GO a possible candidate for biological application. AgNP-GO composites show vigorous antimicrobial activity against Gram-positive and Gram-negative bacteria. Another component to further increase AgNP stability is a biocompatible shell polymer, such as chitosan, further increasing its antimicrobial potency.

The strong synergy between these components makes this composite possibly useful against resistant bacteria[14]. However, not much literature has discussed the impact of this composite in treating resistant UTIs. This literature review aims to present the use of silver, GO, and chitosan to combat uropathogenic resistance, individually or in combination. This literature review is created by reviewing the latest literature sources with the potential for effective UTI treatment, particularly in the rise of resistant pathogens. Firstly, a literature search using the search engine Pubmed, EBSCO-Host, and Google Scholar is done with the combination keywords "new treatment," "urinary tract infection," "bacteria"', "antimicrobial," "resistance," "chitosan," "silver nanoparticle," and "graphene oxide." Boolean operators, AND/OR/NOT, parentheses, asterisks, and quotation marks were used to increase specificity and sensitivity. The inclusion criteria are as follows: articles published in English in the last ten years (2013-2023), aligned with the review question, and are within the evidence level of 1-5. Articles are excluded if the material combination is irrelevant and resistant bacteria are not mentioned or are not common uropathogens. Due to the scarcity of studies on this particular topic, we allowed some leniency toward these criteria. We found 37 original articles, consisting of 33 *in vitro,* 3 *in vivo,* and 1 multicentre cohort study. Most current evidence was experimental studies explaining the potential of chitosan, Ag/AgNP, and GO as a potential antimicrobial against pathogens individually or as a combination of two or three materials. We then searched for other literature on the mechanism of action of the three components of this composite, either as individual ingredients or composites. We also reviewed the pharmacology of this hybrid, finding material drawbacks and assessing the feasibility of its use as an antimicrobial against resistant uropathogens. Synthesis is then created out of these sources, with supporting literature.

2. SUMMARY OF REVIEW FINDINGS

Through literature review, 37 studies were found, divided into three study designs, as follows: (1) 32 *in vitro* studies[14-45], (2) 3 animal studies[46-48], and 1 multicenter cohort study[49]. A summary of the literature reviewed in this study can be reviewed below (see Table 1).

2.1. The potential of silver (Ag) and its nanoparticle (AgNP) as antimicrobials

Silver (Ag) is a precious metal renowned in medicine as an antimicrobial with high potency and low toxicity. Several forms of silver are used pharmacologically, including metallic silver, silver nitrate, silver sulfadiazine, and silver nanoparticle[12,50]. As an antimicrobial, silver has several mechanisms of action. A study tested the impact of Ag^+ ions and AgNO3 on *E. coli* ATCC 23282 and *Staphylococcus aureus* strain ATCC 35696 using a transmission electron microscope (TEM) and X-ray microanalysis. It was found that exposure to silver causes the cell membrane to shrink relative to the cell wall and conglomerated DNA (deoxyribonucleic acid) materials to condense, marked by an increase of phosphorus in the central part of the electron-light region. In the end stage, the homeostatic mechanisms of bacterial cells failed due to the destruction of cellular integrity, marked by electron-dense cell granules and loss of electron-light regions marking the destruction of the cytoskeleton and condensed DNA, causing an inability to

Table 1. A summary of studies related to using silver, silver nanoparticles, chitosan, and graphene oxide as antibacterial agents individually or in combination.

Materials	Author, Year	Design	Sample	Findings
Chit-AgNP-GO	Marta et al. 2015[14]	*In vitro*	Two strains of MRSA: UCLA8076 and 1190R.	- A Chit-AgNP-GO hybrid was created in three different proportions of chitosan to AgNP-GO (1:2, 1:4, and 1:8). - The hybrid with the ratio of 1:4 had an optimal MIC of 1.09 µg/mL Ag+1.35 µg/mL GO, and MBC of 3.28 µg/mL Ag+4.05 µg/mL GO for both MRSA strains. - The result was compared to GO alone, Chit-GO, and AgNP-GO.
	Khawaja et al., 2018[28]	*In vitro*	2 Gram-positive (*S aureus* and *S. mutans*) and 4 Gram-negative (*E. coli, S. typhi, K. pneumoniae,* and *P. aeruginosa*).	- GO, Chit-GO, Ag-GO, and Chit-Ag-GO composites were used against both Gram-positive and Gram-negative bacteria. - The largest inhibition zone was found to be done by Chit-Ag-GO. - Chit-Ag-GO had the lowest MIC value (about 7+0.32 to 10+0.32 µg/mL depending on species).
	Pounraj et al. 2018[34]	*In vitro*	*E. coli* and *B. subtilis* (strains were not specified).	- Chit-GO, AgNP-GO, Chit-GO, and Chit-AgNP-GO composites have anti-biofilm activity on two Gram-negative bacteria in planktonic and biofilm forms. - However, composites laced with AgNP provide contact-killing capabilities. - Comments: More applicable for creating biofilm-proof medical equipment, i.e., Foley catheters.
	Su et al. 2021[45]	*In vitro*	*E. coli* and *S. aureus* colonies (strains were not specified).	- The antibacterial activities of three composites: Chit/RB-AgNP, Chit/RB-AgNP/GO-0.5, and Chit/RB-AgNP/GO-1.5, were tested. - In 24 hours, the inhibition zones of the three composites for *S. aureus* and *E. coli,* respectively, were 0.82 cm and 0.75 cm vs. 0.82 cm and 0.80 cm vs. 0.90 cm and 0.85 cm. Chitosan alone as a control provided no antibacterial benefit. - The combination of chitosan, AgNP, and GO is proven useful for its antibacterial properties.
Ag-GO and AgNP-GO	de Faria et al. 2013[25]	*In vitro*	*B. cereus* ATCC 14579, *S. aureus* ATCC 1901, *C. fimi* NCTC 7547, *L. monocytogenes* ATCC 6477, *E. coli* ATCC 8739, *S. typhimurium* ATCC 14078, *S. enteritidis* ATCC 13076, and *P. aeruginosa* from food isolate.	- The Ag-GO composite inhibited *P. aeruginosa* at an MIC of 2.5-5 µg/mL but did not confer anti-bactericidal effects. - GO alone is incapable of inhibiting bacteria on the diffusion disc.
	Moraes et al. 2015[32]	*In vitro*	MRSA N315, *S. aureus* ATCC 29213, *E. faecalis* 29212, *E. coli* ATCC 25922, *A. baumannii* ATCC 19606, *S. typhimurium* LT2, and *P. aeruginosa* ATCC 27853.	- The Ag-GO composite was a potent bactericidal agent against the pathogens, usually found nosocomially, with an MIC ranging from 15-30 µg/mL and MBC from 15-60 µg/mL. - GO alone was only capable of inhibiting these bacteria at over the concentration of 60 µg/mL.
	Marta et al. 2015[14]	*In vitro*	Two strains of MRSA: UCLA8076 and 1190R.	- The AgNP-GO composites formed had a slightly less effective MIC of 1.90 µg/mL Ag+1.5 µg/mL GO.

Abbreviation: *A. baumannii*: *Acinetobacter baumannii*; *A. franciscana*: *Artemia franciscana*; *A. fumigatus*: *Aspergillus fumigatus*; *A. niger*: *Aspergillus niger*; Ag: silver; AgO_2NP: silver oxide nanoparticle; AgNP: silver nanoparticle; *B. cereus*: *Bacillus cereus*; *B. licheniformis*: *Bacillus licheniformis*; *B. subtilis*: *Bacillus subtilis*; BIC: biofilm inhibitory concentration; *C. albicans*: *Candida albicans*; *C. fimi*: *Corynebacterium fimi*; *C. neoformans*: *Cryptococcus neoformans*; Chit: Chitosan; *E. cloacae*: *Enterobacter cloacae*; *E. coli*: *Escherichia coli*; *E. cloacae*: *Enterobacter cloacae*; *E. faecium*: *Enterococcus faecium*; ESBL: extended spectrum beta-lactamase; GO: graphene oxide; GO-0.5: 0.5 wt% GO; GO-1.5: 1.5 wt% GO; HMW: high molecular weight; ID50: median inhibitory dose; *K. pneumonia*: *Klebsiella pneumoniae*; *K. rhizophila*: *Kocuria rhizophila*; *L. monocytogenes*: *Listeria monocytogenes*; LMW: low molecular weight; *M. morgagnii*: *Morganella morganii*; MBC: minimum bactericidal concentration; MDR: multidrug-resistant; MIC: minimum inhibitory concentration; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; NP: nanoparticle; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. mirabilis*: *Proteus mirabilis*; *P. sanguinolentus*: *Portunus sanguinolentus*; *P. vulgaris*: *Proteus vulgaris*; PVA: polyvinyl alcohol; RB: raspberry-like; rGO: reduced GO; *S. aureus*: *Staphylococcus aureus*; *S. enteritidis*: *Salmonella enteritidis*; *S. epidermidis*: *Staphylococcus epidermidis*; *S. marcescens*: *Serratia marcescens*; *S. mutans*: *Streptococcus mutans*; *S. pyogenes*: *Streptococcus pyogenes*; *S. racemosum*: *Syncephalastrum racemosum*; *S. saprophyticus*: *Staphylococcus saprophyticus*; *S. typhi*: *Salmonella typhi*; *S. typhimurium*: *Salmonella enterica* serovar *Typhimurium*; UPEC: uropathogenic *Escherichia coli*.

Table 1. A summary of studies related to using silver, silver nanoparticles, chitosan, and graphene oxide as antibacterial agents individually or in combination. (cont.)

Materials	Author, Year	Design	Sample	Findings
Ag-GO and AgNP-GO	Khawaja et al., 2018[28]	*In vitro*	2 Gram-positive (*S aureus* and *S. mutans*) and 4 Gram-negative (*E. coli, S. typhi, K. pneumoniae,* and *P. aeruginosa*).	- Ag-GO decently inhibited both Gram-positive and Gram-negative bacteria, with MIC ranging from 15±0.21 to 25±0.35 µg/mL, depending on the species.
	Chen et al. 2019[21]	*In vitro*	Hospital MDR *E. coli* strains (no detailed explanation).	- Ag-GO, GO and AgNP mixture, GO, and AgNP were synthesised and tested against two MDR *E. coli* strains. - GO-Ag composites showed the lowest MIC (4 µg/mL) against the two strains.
	de Saravia, et al. 2019[26]	*In vitro*	*P. aeruginosa* PAO1, *E. coli* ATCC11229, *Acinetobacter* spp. KM349193, *B. cereus* ATCC10876, *Staphylococcus.* spp. (strain unknown), and *K. rhizophila* ATCC9341.	- Two varieties of AgNP-GO: in-situ and ex-situ, were made. - Depending on the species, ex-situ AgNP-GO provided the largest inhibition zone (13-21 mm). AgNP-GO dramatically decreased the population density of *P. aeruginosa* when applied as a protective coating, compared to AgNP alone.
	Cobos et al., 2020[22]	*In vitro*	3 bacteria (*E. coli*. *P. aeruginosa*, and *S. aureus*) and 1 yeast (*C. albicans*).	- Four types of AgNP-GO composites were synthesised with variations in temperature and silver substrate concentration. - These showed antimicrobial properties against three bacteria species and a medically significant yeast.
Chit-GO	Marta et al. 2015[14]	*In vitro*	Two strains of MRSA: UCLA8076 and 1190R.	- The Chit-GO formed had an MIC of 2.25 µg/mL GO to both MRSA strains, compared to chitosan alone, which inhibited MRSA at an MIC of >7.5 µg/mL.
	Khawaja et al., 2018[28]	*In vitro*	2 Gram-positive (*S aureus* and *S. mutans*) and 4 Gram-negative (*E. coli, S. typhi, K. pneumoniae,* and *P. aeruginosa*).	- Chit-GO showed a mediocre inhibition zone diameter and an MIC on par with GO alone in inhibiting all six bacteria species (25±0.21 vs. 45±0.30 µg/mL, depending on species).
	Maruthupandy, et al. 2020[31]	*In vitro*	Biofilm of *P. aeruginosa* and *K. pneumoniae*. *A. franciscana* as a model for eukaryotic organisms.	- Chit-GO composites inhibited up to 94% and 92% of the biofilm formation of *P. aeruginosa* and *K. pneumoniae*, at a 40 µg/mL concentration. - Comments: Chit-GO composites were shown to be toxic to *A. franciscana* and, therefore, towards eukaryotic (including human) cells at a concentration of 70 µg/mL.

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Chit-GO	Alfuraydi et al. 2022[15]	*In vitro*	*K. pneumoniae* ATCC 13883, *P. mirabilis* ATCC 12453, *E. coli* ATCC 1175, *P. aeruginosa* ATCC 10145, *A. baumannii* ATCC 19606, *B. subtilis* ATCC 6051, *S. epidermidis* ATCC 12344, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 12344, MRSA ATCC-BAA-1720, *S. pyogenes* ATCC 12344, *A. fumigatus* ATCC 9197, *S. racemosum* ATCC 14833, *A. niger* ATCC 6275, *C. neoformans* ATCC 66031, and *C. albicans* ATCC 18804.	- Hydrogels using polyvinyl alcohol (PVA) and chitosan were used with different weight ratios and then cross-linked with trimellitic anhydrate isothiocyanate. Additionally, composites were tested by preparing 3:1 chitosan: PVA hydrogel (H31) with a differing concentration of AgNP. - PVA itself showed no inhibitory properties towards tested pathogens. Between all PVA and chitosan hydrogel, higher inhibitory properties were shown by hydrogel with higher chitosan: PVA weight ratio (3:1>1:1>1:3). However, even the 3:1 hydrogel still has lower potency than that of vancomycin and amphotericin B. The hydrogels generally showed higher inhibitory activity against Gram-negative bacteria. - Adding AgNP to the 3:1 hydrogel (H31) enhances inhibitory activity. The optimal concentration of AgNP was found to be 3%. On Gram-negative bacteria, MIC values for H13 with 3% AgNP were 10, 6.88, 3.75, 2.25, and 1.87 µg/mL for *K. pneumonia, P. mirabilis, E. coli, P. aeruginosa, and A. baumannii* respectively. On Gram-positive bacteria, the MIC values were 2.38, 1.5, 1.0, 0.63, and 0.13 µg/mL for *S. pyogenes, S. aureus, S. epidermidis,* MRSA*, and B. subtilis.* On fungi, the MIC values were 6.25, 2.13, 1.5, 1, and 0.75 µg/mL for *S. racemosum, C. neoformans, A. niger, A. fumigatus,* and *C. albicans,* respectively. No significant enhancements were observed with the use of 5% AgNP.
	Alzubaidy et al. 2022[18]	*In vitro*	*E. coli.*	- Chit-GO composites effectively inhibited *E. coli*, with an ID50 of 1 mg/ml inhibiting 80% of *E. coli* in urine. - Chit-GO did not cause significant hemolysis at 1 mg/mL but did at concentrations of 5 and 10 mg/mL.
Chit-Ag and Chit-AgNP	Rajivgandhi et al. 2019a[36]	*In vitro*	ESBL-producing *P. aeruginosa.*	- AgNP and Chit-AgNP composites were tested against ESBL-producing *P. aeruginosa*, and can inhibit up to 76% and 92% of bacterial growth, respectively, at an MIC of 80 µg/mL and 50 µg/mL each. - Comments: These composites can also deform and destroy bacteria cells.
	Samanta et al. 2022[42]	*In vitro*	MDR uropathogenic *E. coli* (MLD-2 strain) and *S. aureus* (MLD-4 strain).	- The MIC and MBC values of Ag-Chit NP for the *E. coli* strain were both 12.5 µg/mL. The MIC and MBC values of Ag-Chit NP for *S. aureus* were 25 and 50 µg/mL, respectively. - Ag-Chit NP did not show significant hemolytic properties at MIC values. There were no major hemolytic properties up to a concentration of 50 µg/mL.
Chitosan	Paul et al. 2014[33]	*In vitro*	*E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. mirabilis* ATCC 29906, and clinical strains of *E. coli*, *K. pneumoniae*, and *P. mirabilis.*	- Chitosan inhibited *E. coli* and *P. mirabilis* at a minimum concentration of 50 µg and *K. pneumoniae* at a minimum of 100 µg. - Linear correlation was found between chitosan concentration and the size of the inhibition zone.
	Blango, et al. 2014[46]	Animal (*in vivo*)	UPEC (UTI89) and 7-to-8 weeks old CBA/J mice.	- Chitosan was shown to reduce biofilm production of UPEC (UTI89), lower bacterial concentration, and decrease urothelial invasion and intracellular bacteria when administered via transurethral catheterisation with antibiotics.
	Kumar et al. 2016[29]	*In vitro*	*E. coli* MTCC 729 and *P. mirabilis* MTCC 425.	- Both chitosan and kanamycin in nanoparticle form were immobilised on the surface of a polyurethane urethral stent, and better decreased both bacteria's concentrations compared with an unmodified stent.

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Chitosan	Dragland et al. 2016[27]	*In vitro*	*S. epidermidis* ATCC 35984.	- LMW chitosan was proven to be applicable against *S. epidermidis* and reduces biofilm formation significantly in a concentration-dependent manner.
	Costa et al. 2017[23]	*In vitro*	MRSA strain CCUG 60578 and MSSA strain ATCC 25923, as well as one each MRSA and MSSA strain from clinical settings.	- HMW (624 kDa) and LMW (107 kDa) chitosan inhibited all *S. aureus* strains. In LMW chitosan, MBC was higher for MRSA than MSSA isolates. - There was no significant difference in minimum BIC between HMW and LMW chitosan, but metabolic activity is more easily inhibited by either HMW or LMW chitosan in MRSA than in MSSA.
	Campana et al. 2017[20]	*In vitro*	Clinical isolates of *K. pneumoniae* and *E. coli.*	- Chitosan (50 and 150 kDa) was tested in two pH settings (5.0 and 6.0) against *K. pneumoniae* and *E. coli* in a time-kill study and anti-adhesion assay. - The antibacterial action of chitosan was facilitated by decreased molecular weight and lower pH. Following exposure to chitosan, bacterial viability and population both declined over time. Additionally, chitosan prevented adherence to the catheter.
	Erman et al. 2017[47]	Animal (*in vivo*)	UPEC and 87 mice as animal models.	- Repeated treatment with an 80 µg exfoliant mixture of chitosan (0.5%) with and without ciprofloxacin (unstated dose) for four days intravesically can eliminate UPEC reservoirs and incidence of bacteriuria in an animal model.
	Rubini, et al. 2019[39]	*In vitro*	*P. aeruginosa,* PA01 and clinical strains, as well as clinical strain of *S. marcescens.*	- The extracted chitosan from the marine crab *P. sanguinolentus* significantly inhibited some virulence factors in the laboratorium and clinical strains of *P. aeruginosa* and a clinical strain of *S. marcescens* bacteria. - Compared to commercial chitosan, the extracted chitosan inhibited mature biofilm when put as catheter coating.
	Rubini, et al. 2020[40]	*In vitro*	UPEC.	- Chitosan can inhibit UPEC both as planktonic bacteria and as a biofilm.
rGO	Almamad et al. 2021[16]	*In vitro*	350 urine samples from UTI patients and bacterial isolates: *E. coli, K. pneumoniae, P. aeruginosa, E. cloacae, S. saprophyticus,* and MRSA	- rGO was effective against all tested pathogens with a zone of inhibition of 36, 29, 21, 29, 33, and 19 mm against *E. coli, K. pneumoniae, P. aeruginosa,* MRSA, *E. cloacae,* and *S. saprophyticus,* respectively at the highest concentration tested (10 mg/mL). - Combination with antibiotics also showed an increase in antibacterial activity. The enhancement was especially apparent when combined with amoxicillin/clavulanic acid, cefotaxime, and ceftriaxone against *E. coli, K. pneumoniae, P. aeruginosa,* and *S. aureus*, where no inhibition zone was formed when the antibiotic was used without rGO.
Ag/AgNP	Lederer, et al. 2014[49]	Multicenter cohort	Adults with positive urine culture ≥2 days after admission who underwent Foley catheterisation in seven acute care hospitals with 124-607 beds.	- Comparing standard Foley catheters to ones coated in Ag hydrogel, coated catheters are proven to reduce CA-UTI incidence by 47% and reduce the number of treatment days from 1,165 days with standard catheters to 406 days.
	Alshehri, et al. 2016[17]	*In vitro*	*E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. aureus,* and *P. mirabilis.*	- Hydrogel impregnated with AgNP showed higher antimicrobial activity against *E. coli, K. pneumoniae, P. aeruginosa*, *P. vulgaris*, *S. aureus*, and *P. mirabilis* than the hydrogel alone.

Abbreviation: *A. baumannii*: *Acinetobacter baumannii*; *A. franciscana*: *Artemia franciscana*; *A. fumigatus*: *Aspergillus fumigatus*; *A. niger*: *Aspergillus niger*; Ag: silver; AgO_2NP: silver oxide nanoparticle; AgNP: silver nanoparticle; *B. cereus*: *Bacillus cereus*; *B. licheniformis*: *Bacillus licheniformis*; *B. subtilis*: *Bacillus subtilis*; BIC: biofilm inhibitory concentration; *C. albicans*: *Candida albicans*; *C. fimi: Corynebacterium fimi*; *C. neoformans*: *Cryptococcus neoformans*; Chit: Chitosan; *E. cloacae*: *Enterobacter cloacae*; *E. coli*: *Escherichia coli*; *E. cloacae*: *Enterobacter cloacae*; *E. faecium*: *Enterococcus faecium*; ESBL: extended spectrum beta-lactamase; GO: graphene oxide; GO-0.5: 0.5 wt% GO; GO-1.5: 1.5 wt% GO; HMW: high molecular weight; ID50: median inhibitory dose; *K. pneumonia*: *Klebsiella pneumoniae*; *K. rhizophila*: *Kocuria rhizophila*; *L. monocytogenes*: *Listeria monocytogenes*; LMW: low molecular weight; *M. morgagnii*: *Morganella morganii*; MBC: minimum bactericidal concentration; MDR: multidrug-resistant; MIC: minimum inhibitory concentration; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; NP: nanoparticle; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. mirabilis*: *Proteus mirabilis*; *P. sanguinolentus*: *Portunus sanguinolentus*; *P. vulgaris*: *Proteus vulgaris*; PVA: polyvinyl alcohol; RB: raspberry-like; rGO: reduced GO; *S. aureus*: *Staphylococcus aureus*; *S. enteritidis*: *Salmonella enteritidis*; *S. epidermidis*: *Staphylococcus epidermidis*; *S. marcescens*: *Serratia marcescens*; *S. mutans*: *Streptococcus mutans*; *S. pyogenes*: *Streptococcus pyogenes*; *S. racemosum*: *Syncephalastrum racemosum*; *S. saprophyticus*: *Staphylococcus saprophyticus*; *S. typhi*: *Salmonella typhi*; *S. typhimurium*: *Salmonella enterica* serovar *Typhimurium*; UPEC: uropathogenic *Escherichia coli*.

Table 1. A summary of studies related to using silver, silver nanoparticles, chitosan, and graphene oxide as antibacterial agents individually or in combination. (cont.)

Materials	Author, Year	Design	Sample	Findings
Ag/AgNP	Shafreen, et al. 2017[43]	*In vitro*	ESBL-producing *E. coli.*	- AgNP was tested against an ESBL-producing strain of *E. coli* with BIC found at 300 ng/mL. - Comments: More suited for applications related to the coating of medical devices (i.e., Foley catheters).
	Dayyoub, et al. 2017[24]	*In vitro*	*P. mirabilis.*	- AgNP and norfloxacin were loaded to polymer films, applied to polyurethane and silicone sheets, and compared to standard polyurethane and silicone sheets against *P. mirabilis*. - The experiment revealed that the coated sheets could resist encrustation for 50 days *in vitro* encrustation model.
	Mala et al. 2017[48]	Animal (*in vivo*)	Normal flora from mice urinary tract.	- Foley catheters were impregnated with AgNP plus amikacin and nitrofurantoin, and they were compared with catheters impregnated with antibiotics alone in an anti-adhesion study for two years. - Catheters impregnated with antibiotics alone, embedded in mice, only exhibited 25% of antimicrobial activity after two years against the mice's normal flora. - Catheters impregnated with antibiotics and AgNP exhibited 90% antimicrobial activity after two years. The blood, liver, and kidney functions of all mice involved are normal.
	Srinivasan, et al. 2018[44]	*In vitro*	*S. marcecens* and *P. mirabilis.*	- AgNP works against the biofilm-associated virulence factors of *S. marcescens* and *P. mirabilis* by inhibiting quorum-sensing-mediated virulence factors such as prodigiosin, protease, biofilm formation, exopolysaccharides, and hydrophobicity. - AgNP was also non-toxic when given to human cells (human mononuclear and lung epithelial cells).
	Ashmore et al. 2018[19]	*In vitro*	*E. coli.*	- The study tested AgNP, polymer-coated 99% AgNP, and polymer-coated 10% AgNP against *E. coli*. MIC respectively was 0.621 mg/ml, 0.312 mg/ml, and 0.312 mg/ml
	Lopez-Carrizales, et al. 2018[30]	*In vitro*	Uropathogens (*E. faecium*, *S. aureus*, *A. baumannii*, *E. cloacae*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *M. morgagnii*).	- AgNP is tested alone and in combination with amikacin and ampicillin, respectively, against several different clinical isolates of uropathogenic agents. - For all clinical isolates, the MIC of AgNP ranged between 4-16 µg/mL. - Combined with antibiotics, AgNP can decrease the MIC (compared to antibiotics alone) by 4-32 fold.
	de Saravia, et al. 2019[26]	*In vitro*	*P. aeruginosa* PAO1, *E. coli* ATCC11229, *Acinetobacter* spp. KM349193, *B. cereus* ATCC10876, *Staphylococcus.* spp. (strain unknown), and *K. rhizophila* ATCC9341.	- AgNP alone created an inhibition zone on the discs of most bacteria. Gram-positive bacteria were less affected than Gram-negative bacteria.
	Rajivgandhi et al. 2019b[37]	*In vitro*	Coagulase-negative MRSA.	- AgNP could inhibit coagulase-negative MRSA with an MIC of 55 µg/mL. AgNP was also able to stop biofilm production at the same concentration.
	Sajjad et al. 2019[41]	*In vitro*	ESBL-producing UPEC.	- 10 µg/mL AgO_2-NP was loaded with 10 µg/mL ceftriaxone and used against ESBL-producing UPEC. - It yielded a significantly larger inhibition zone of ESBL-producing UPEC (26.4±2 mm) compared with ceftriaxone alone (10.0±1 mm).

Abbreviation: *A. baumannii*: *Acinetobacter baumannii*; *A. franciscana*: *Artemia franciscana*; *A. fumigatus*: *Aspergillus fumigatus*; *A. niger*: *Aspergillus niger*; Ag: silver; AgO_2-NP: silver oxide nanoparticle; AgNP: silver nanoparticle; *B. cereus*: *Bacillus cereus*; *B. licheniformis*: *Bacillus licheniformis*; *B. subtilis*: *Bacillus subtilis*; BIC: biofilm inhibitory concentration; *C. albicans*: *Candida albicans*; *C. fimi:* *Corynebacterium fimi*; *C. neoformans*: *Cryptococcus neoformans*; Chit: Chitosan; *E. cloacae*: *Enterobacter cloacae*; *E. coli*: *Escherichia coli*; *E. cloacae*: *Enterobacter cloacae*; *E. faecium*: *Enterococcus faecium*; ESBL: extended spectrum beta-lactamase; GO: graphene oxide; GO-0.5: 0.5 wt% GO; GO-1.5: 1.5 wt% GO; HMW: high molecular weight; ID50: median inhibitory dose; *K. pneumonia*: *Klebsiella pneumoniae*; *K. rhizophila*: *Kocuria rhizophila*; *L. monocytogenes*: *Listeria monocytogenes*; LMW: low molecular weight; *M. morgagnii*: *Morganella morganii*; MBC: minimum bactericidal concentration; MDR: multidrug-resistant; MIC: minimum inhibitory concentration; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; NP: nanoparticle; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. mirabilis*: *Proteus mirabilis*; *P. sanguinolentus*: *Portunus sanguinolentus*; *P. vulgaris*: *Proteus vulgaris*; PVA: polyvinyl alcohol; RB: raspberry-like; rGO: reduced GO; *S. aureus*: *Staphylococcus aureus*; *S. enteritidis*: *Salmonella enteritidis*; *S. epidermidis*: *Staphylococcus epidermidis*; *S. marcescens*: *Serratia marcescens*; *S. mutans*: *Streptococcus mutans*; *S. pyogenes*: *Streptococcus pyogenes*; *S. racemosum*: *Syncephalastrum racemosum*; *S. saprophyticus*: *Staphylococcus saprophyticus*; *S. typhi*: *Salmonella typhi*; *S. typhimurium*: *Salmonella enterica* serovar *Typhimurium*; UPEC: uropathogenic *Escherichia coli*.

Table 1. A summary of studies related to using silver, silver nanoparticles, chitosan, and graphene oxide as antibacterial agents individually or in combination. (cont.)

Materials	Author, Year	Design	Sample	Findings
Ag/AgNP	Rodriguez-Serrano, et al. 2020[38]	*In vitro*	UPEC.	- AgNP can inhibit a strain of UPEC with an MIC of 25 µg/mL and a BIC of 7.5 µg/mL.
	Rahuman, et al. 2021[35]	*In vitro*	*S. aureus*, *E. coli*, and *P. aeruginosa.*	- AgNP exhibit inhibitory properties with an MIC of 60 µg/mL, 40 µg/mL, and 40 µg/mL for *E. coli*, *S. aureus*, and *P. aeruginosa,* respectively. - AgNP was also found to be synergistic with antibiotics, especially shown by treatment with trimethoprim; the zone of inhibition was increased in area by 2.3-fold, 1.04-fold, and 3.84-fold for *S. aureus*, *E. coli*, and *P. aeruginosa,* respectively. - The highest concentration of AgNP (160 µg/mL) shows inhibitory activity against biofilm, up to 85.8±1.450%, 82.8±1.83%, and 71.4±1.25% for *P. aeruginosa*, *S. aureus*, and *E. coli* respectively.

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reproduce. The results are similar in both bacteria species, with only slightly stronger cellular integrity, less electron-dense granules, and darker electron-light regions in *S. aureus*[51]. Another impact is the destruction of flagella and fimbriae, causing an inability to move and exchange genes, causing the bacteria being active but not culturable (ABNC)[52].

Based on the experiment, it is known that silver interacts with DNA and both structural and functional proteins[51]. There is a conglomeration of Ag^+ ions inside bacterial DNA structures. Destruction of cellular integrity also occurs due to the change of a thiol's sulfhydryl (S-H) bonds into S-Ag bonds. The sulfhydryl bonds are essential because it forms the basis of several enzymatic functional chains, such as enzymes in the respiration chains, i.e., nicotinamide adenine dinucleotide phosphate (NADH)-dehydrogenase I and II[51,53]. Due to the destruction of these enzymes, there is a loss of proton motive force through the bacterial cell membrane, uncoupling of the respiration chain, an inefficiency of electron transport towards oxygen, and an increase in the production of reactive oxygen species (ROS)[53-54]. Other enzymes affected by silver are those with [4Fe-4S] bonds, such as the various dehydratases composing the electron transport chain[55]. Quantitatively, the antimicrobial efficacy of Ag^+ ions is known to be potent enough to eliminate uropathogens significantly; The addition of Ag^+ ions destroys 80-100% of bacteria, depending on the observing techniques[53].

Pathogenic resistance to silver may happen when used as an antimicrobial for a long time. Nevertheless, due to its multiple mechanisms of action, the spontaneous mutation rate of bacteria towards silver-based antimicrobial substances is lower than other antimicrobials, and resistance is less likely to happen[50].

Silver is often used as a coating for Foley catheters to prevent or treat catheter-associated UTIs (CA-UTI). In a cohort study on seven hospitals with a three-month minimum of standard catheter use vs Ag hydrogel catheters, Ag hydrogel catheters are proven to reduce CA-UTI incidence and number of treatment days[49]. Recognition of the antimicrobial effects of silver has been long-standing, and according to one meta-analysis reviewing eight clinical trials of silver alloy on catheters, there is a more significant protective effect on bacteriuria (recorded OR 0.79, 95%CI: 0.56-1.10) but with an increased cost of 6 USD[56]. A systematic review shows that silver alloy-coated catheters are worth 3.56-9.71 USD, 80-130% higher than standard catheters. However, using silver alloy-coated catheters can reduce the chances of infection in the short term[57].

Several studies assessed that silver has a practical antimicrobial effect on the concentration of 10^{-9} to 10^{-6} M[58]. The stability of silver gets lower when exposed to ions in the human body, such as Cl^- ions, which can cause a salt reaction and deposition. Therefore, silver is commonly combined with other substances which stabilise it or are synthesised as nanoparticles to alter its pharmacokinetics.

Silver is sometimes formulated as nanoparticles (AgNP) to increase effectiveness, which is effective due to its small size and high zeta potential. Small molecular size allows AgNP to penetrate bacterial outer and inner membranes, especially in Gram-negative bacteria. Thus, AgNP can disrupt porin and lipopolysaccharide components in the cytoplasm and destabilise the outer membrane[59]. It then bonds itself into the bacterial cell walls, causing dephosphorylation of important peptide substrates. This process modulates cellular signals in bacteria [60]. AgNP also perforates bacterial cell walls and enters the cell, condensing genetic material and coagulating it with cytoplasm, causing cytoplasmic leakage and bacterial cell death[61]. A smaller size and increased surface area increased efficacy in AgNP compared to silver. The electrostatic property of AgNP is also a vital mechanism for its antibacterial and antibiofilm activity. The positive charge of AgNP interacts with bacterial enzymes and proteins, inhibiting their activity. This electrostatic interaction also disturbs biofilm formation, degrades EPS, and breaks biofilm mats. Finally, AgNP may interact with bacterial ribosomes, causing inhibition in the translation process[35].

Although there are a few disagreements regarding the usage of AgNP vs Ag^+ ions, it is agreed upon that the concentration scale is different. AgNP is more effective than Ag alone on the nanomolar scale, while Ag^+ ions react on the micromolar scale. However, Ag+ ions and AgNP are broad-spectrum antimicrobial agents, effective against Gram-positive and Gram-negative bacteria[62]. The released Ag^+ ions produce reactive oxygen species (ROS), which further destroy the bacterial cell membrane by increasing its permeability[15].

Several studies have shown AgNP effectiveness in biofilms. Research on the uropathogenic *Enterococcus faecalis* on dentin slides in four weeks using AgNP solution vs AgNP gel prefers the usage of AgNP gel as it is more effective at disrupting the structural integrity of biofilm and killing bacteria inside[63]. Research on *Pseudomonas aeruginosa* and *S. epidermidis* in an agar medium also shows that AgNP can significantly create an inhibition zone and reduce biofilm production of both bacteria[64]. In another trial, *P. aeruginosa* no longer grows after a treatment of 10 mg/L of AgNP. Other uropathogens, such as *S. aureus*, are inactive 48 hours after receiving 5 mg/L of AgNP. Meanwhile, *E. coli* can also be inactivated 48 hours after treatment with 10 mg/L of AgNP[61]. This vigorous antibacterial activity towards biofilm has led AgNP (as well as Ag^+) to be commonly used as an antibacterial coating on invasive devices, such as Foley catheters. Studies in Table 1 discussing this application are Alshehri et al.,[17] Shafreen et al.,[43] Mala et al.,[48] and Ashmore et al.[19] All studies agree that AgNP

inhibits biofilm structural integrity and kills bacteria released from the aggregate structures.

As a xenobiotic metal, silver can cause delayed hypersensitivity and allergies in those with predispositions, although the risk is not yet studied in depth[62]. Other studies suggested that a cytotoxic effect occurred with exposure to AgNP. AgNP can reduce cellular adenosine triphosphate (ATP), leading to mitochondrial dysfunction and increased ROS production. This mechanism will lead to DNA damage and may cause a stop to the cell cycle in the G2/M phase, which might be clinically harmful. In AgNP, the toxicity depends on particle size, as proven by a cell proliferation assay. It should also be noted that the LD50 (median lethal dose) regarding the mentioned cytotoxicity in the study uses various cell lines from various non-standardised sources; therefore, the LD50 could differ in different cell lines[65]. The use of silver can also cause argyria, a harmless deposition of silver manifesting as cosmetic changes without any significant damage or dysfunction to tissue[62].

The pharmacokinetic process of AgNP in the human body has also been studied. AgNP can be administered through injection and excreted through urine and faeces. The highest faecal concentration occurs two days after injection. In the body, AgNP is distributed from the blood to the inner organs, with a distribution pattern not changing even after 30 days[66].

2.2. The potential of graphene oxide (GO) as an antimicrobial

Graphene oxide (GO) is graphene sheets with a carboxylic group on its ends, epoxide and hydroxyl groups on its basal plane, and hexagonal crystals of carbon atoms[67-68]. The material is small but with a high density of functional groups, allowing for easier deposition on bacteria[67]. GO can treat various biofilm-producing organisms, including Gram-positive and Gram-negative bacteria[68].

GO as coating allows less bacterial contact to the orthogonal ends of GO, increasing interaction with the basal plate. An oxidative mechanism then eliminates microbes. The increasingly smaller GO sheets increase defect density and oxidative stress[68]. As a suspension, GO uses a cell-trapping mechanism; it coats bacteria and prevents proliferation. In this mechanism of action, larger GO sheets are more effective. However, trapped bacteria can replicate once moved out of the suspension, which shows that GO is a bacteriostatic agent, not a bactericidal one[68]. The orthogonal edges of GO are sharp and can physically disrupt cell membranes, causing electrolyte and RNA leakage in bacteria.[21,68]. The structural damage generates ROS, further damaging the bacteria, and facilitates the passage of antibiotic agents into the bacteria[16]. GO can also absorb amino acids and prevent nutrient absorption, disrupting bacterial metabolism[21].

Combined with AgNP, the two components can form a composite particle (AgNP-GO) with synergistic effects. GO can help increase the surface area of interaction between AgNP and bacterial cells[25,32]. AgNP is embedded on GO sheets and is ejected when GO adheres to bacterial cells[32]. The efficiency of AgNP is increased by compositing it with GO[25,32]. One study evaluated the development of MRSA (methicillin-resistant *S. aureus*) and *E. coli* colonies when given GO and AgNP-GO compared to the negative control. In contrast to colonies that received GO, those given AgNP-GO experienced a considerable replication impediment[32]. A similar evaluation was also made with the application of 2.0 µg/mL of AgNP-GO on *P. aeruginosa*, which was capable of eradicating the colonies, while similar concentrations of GO have no discernible impact on colony size and replicability[25]. Studies by Chen et al.[69] and Su et al.[45] also show the flexibility and antibacterial potential associated with AgNP-GO[45,69].

Almamad et al.[16] show that not only reduced GO (rGO) exhibit an inhibitory effect towards both Gram-positive and Gram-negative bacteria, but it also enhances the performance of common antibiotics. A combination of rGO with 30 µL of 1 mg/mL rGO solution with amikacin, cefotaxime, and cefixime on multidrug-resistant (MDR) bacteria isolates created an inhibition zone up to 13 mm when no inhibition zone was made with the antibiotics alone[16]. However, no clinical trials have been developed for GO, and the data given so far are from *in vitro*, animal model, and observational studies. All of them, however, show promising antimicrobial effects. A trial with A549 cells has also proven that GO does not confer a cytotoxic effect on human cells, although it can cause increased oxidative stress in higher doses[70]. GO can be administered intravenously, and trials in mice show accumulation in the liver and renal endoplasmic reticulum, which dissipates slowly. The excretion of GO is done through faeces and urine[71].

2.3. The potential of chitosan as an antimicrobial

Chitosans are a group of deacetylated chitin compounds. The potential of chitosan in medicine was first reported in 1979 when shrimp chitosan was found to be fungicidal. Further research discovers an antimicrobial effect on Gram-positive and Gram-negative bacteria and its safety for use in humans. Chitosan possesses several antimicrobial mechanisms. The positively charged amino groups in chitosan can interact with bacterial cell membranes, causing cellular leakage. It also interacts with the phospholipid bilayer, changing its permeability and ending with cellular leakage[72].

Costa et al.[23] suggest that the antimicrobial effect of chitosan depends on its molecular weight, degree of polymerisation, and pH, since these factors affect its charge density (degree of polymerisation and pH) and

solubility (degree of polymerisation and molecular weight)[23,73]. A trial using both high molecular weight (HMW) and low molecular weight (LMW) chitosan was done against MRSA and methicilin-sensitive *S. aureus* (MSSA) isolates, and it was discovered that chitosan could significantly decrease both planktonic and biofilm production of bacteria[23]. Conversely low-viscosity, LMW chitosan inhibit *S. epidermidis* biofilm. The bacteriostatic effect is found at a concentration of 0.003% w/v, and the bactericidal effect is at ≥0.005% w/v[27]. Other uropathogens such as *E. coli*, *Proteus* spp., and *Klebsiella* spp. are treated with chitosan with a molecular weight of 72.16 kilodaltons (kDa) with diffe-rent concentrations. *E. coli* and *P. mirabilis* are inhibited at lower MIC than *K. pneumoniae*[33].

Chitosan is also used to facilitate antimicrobial transfer through tissue or into bacterial cells, carrying various substances such as silver[74], antimicrobial peptides[75], propolis[76], or even antibiotics such as ciprofloxacin, gentamicin, and chlortetracycline[77]. It is not only a transporting vessel; it can also enhance drug effectiveness by providing a slow-release effect and increasing the absorption of certain drugs. It is also effective on various bacteria with various antibiotic resistance levels[74-77]. Other applications include coating invasive devices such as Foley catheters, attributing to their anti-biofilm properties[39-40], and administration as an intravesical antibacterial agent, whether with or without antibiotics[29,46-47].

Biofilm inhibition is another critical feature of chitosan. A study by Alzubaidy et al.[18] shows inhibition of *E. coli* biofilm. The anti-biofilm activity is due to the polycationic nature of chitosan, which interacts with EPS (extracellular polymeric substances), protein, and bacterial DNA, preventing biofilm production. However, the study used chitosan linked with GO (Chit-GO) and did not evaluate the activity of chitosan by itself[18]. A study by Alfuraydi et al.[15] evaluated the activity of pure chitosan against biofilm but found its inhibitory action to be poor, with a MIC as high as 1,000 µg/mL. Chitosan was only shown to inhibit biofilm when blended with trimellitic anhydride isothiocyanate, with or without blending with polyvinyl alcohol (PVA)[15].

In addition to its antibacterial and anti-biofilm action, the study by Alfuraydi et al.[15] shows that chitosan can also inhibit the growth of medically significant fungi, such as *Aspergillus fumigatus*, *Syncephalastrum racemosum*, *A. niger*, *Cryptococcus neoformans*, and. It is posited that this antifungal activity is mediated through the modification of cell wall morphology and chelation of metal ions necessary for growth, similar to its inhibition of bacterial growth. Additionally, chitosan inhibits fungal growth by penetrating the hyphae and disrupting enzyme and protein activities[15].

In combination with AgNP, chitosan was tested against ESBL (extended-spectrum beta-lactamase)-producing *P. aeruginosa* and could inhibit a significant amount of bacterial growth with low MIC. It also showed some bactericidal activity[36]. Another study confirms its efficacy against MDR *E. coli* and *S. aureus* with even lower MIC[42]. Composited with GO, chitosan was also shown to be a somewhat effective bacteriostatic agent, more than chitosan alone. Another study even shows that Chit-GO is effective on fungi when prepared as a hydrogel combined with polyvinyl alcohol (PVA)[15]. However, it might be toxic to eukaryotic cells[28,31].

An in-depth animal study on chitosan use shows that it is metabolised in the liver and gut flora and excreted through urine[78]. Chitosan can be administered orally, intravenously, and topically (e.g., mouthwash)[78-79]. Long-term ingestion of chitosan has also been tested for its safety. Low molecular weight (65 kDa) chitosan and its derivatives are tested thoroughly with a mouse model. Chitosan has a minimal hemolytic effect (2-5%), which is induced by heat. It is not hepatotoxic or nephrotoxic and does not change either organ's histomorphology[80]. The hemolytic effect of chitosan has also been studied *in vitro* in combination with GO and AgNP. Chitosan combined with AgNP did not show significant hemolytic activity at MIC[42]. Chitosan combined with GO also did not significantly cause hemolysis at the median inhibitory dose (ID50) for *E. coli*[18]. Chitosan is also a sustainable resource, obtainable from crustacean shell waste.

2.4. The antimicrobial impact of chitosan-silver nanoparticle-graphene oxide hybrid

Synergic hybridisation works well as a method to combat antimicrobial resistance due to its ability to interfere with multiple pathogenic metabolic pathways. We found four relevant studies which documented tests on the combination of the three components mentioned above. An experimental study by Martaa et al.[14] assessed the potential of a hybrid consisting of chitosan, silver nanoparticles, and graphene oxide (Chit-AgNP-GO). This trial compares the hybrid with its components against two strains of MRSA, UCLA 8076 and 1190R. *S. aureus* is one of the most prevalent Gram-positive organisms causing UTIs. The strain UCLA 8076 consists of several subpopulations with high proportions (≥99.9%) of drug-sensitive bacteria and a tiny proportion of drug-resistant bacteria (1 in 10^6 cells), while strain 1190R consists of a homogenous population with high resistance to antibiotics[14].

The material was created by first hybridising the AgNP and GO sheets by embedding AgNP on the GO sheets. Chitosan is then adsorbed in the new composite sheets. This process creates a configuration of AgNP embedded firmly in GO and chitosan adsorbed on its surface—the synthesis procedure is in detail below (see Figure 1)[14].

The hybrid formed (with a ratio of chitosan to AgNP-GO of 1:8) has a more favourable surface potential due

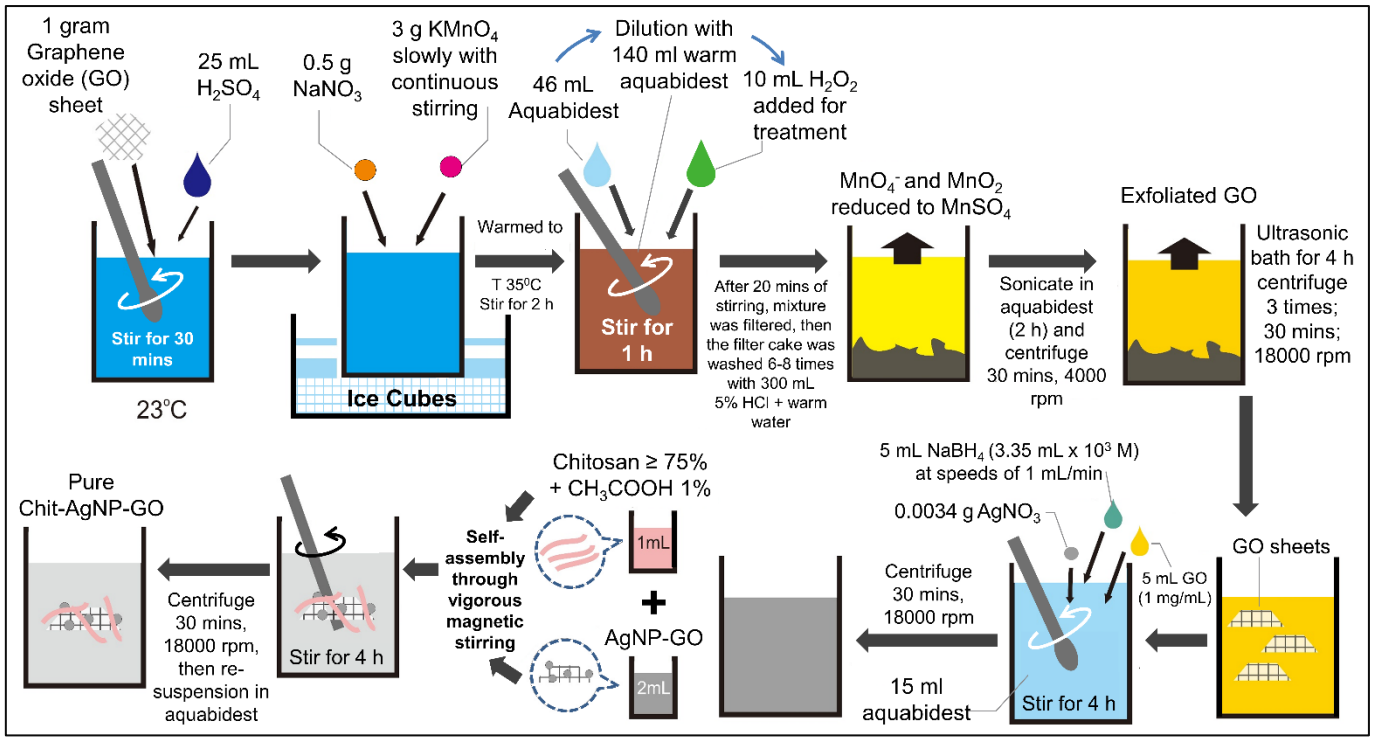

Figure 1. Proposed steps for making the composite Chit-AgNP-GO particles. GO sheets are first embedded with AgNP. This combination is then embedded with Chit. Chit will then adsorb into the surface of the hybrid[14].
Abbreviation: AgNP (silver nanoparticle); $AgNO_3$ (silver nitrate); CH_3COOH (acetic acid); Chit (chitosan); DNA (deoxyribonucleic acid); GO (graphene oxide); $KMnO_4$ (potassium permanganate); $NaNO_3$ (sodium nitrate); MnO_2 (magnesium dioxide); MnO_4 (permanganate); $MnSO_4$ (magnesium sulfate); ROS (reactive oxygen species)

to the addition of chitosan. Without chitosan, the AgNP-GO hybrid has a more negative surface potential. A more positive surface potential, provided by the poly-cations that form chitosan, allows easier adhesion of the hybrid to the negatively charged surface of bacterial cells. Chitosan also interacts with bacteria by neutralising bacterial anionic chemical groups on the cell surface, chelating nutrients necessary for enzymatic activity, and increasing the release of Ag^+ ions from silver nanoparticles[14].

Antimicrobial activity is measured through MIC and MBC. As seen on Table 1, the Chit-AgNP-GO hybrid has a MIC in both ratios (1:4 and 1:8) significantly lower than AgNP-GO alone. The bacteriostatic activity described above is similar for both MRSA strains[14]. According to the study, an appropriate ratio of chitosan and AgNP-GO is required for the hybrid produced to be neither too "thin," causing a lower affinity towards bacterial cell membrane, nor too "thick," inhibiting Ag^+ ion diffusion through the nano-scale contact point[14]. An optimal ratio between chitosan and AgNP-GO to create the best bactericidal effect lies at 1:4. This creates a much lower MBC than the MBC for the individual components of this hybrid. The increase in bactericidal activity can be due to the synergy between the three components. Chitosan facilitates adhesion to bacterial cells, while GO influences liposolubility and cell permeability. AgNP is also a potent antimicrobial that affects particle size and surface area, causing the inactivation of bacterial cell osmosis and oxidative stress[14].

Supporting the combination of these three materials, Khawaja et al.[28] and Pounraj et al.[34] show that similar hybrids are effective against various Gram-positive and Gram-negative bacteria, with a MIC of <10 µg/mL, more efficient than hybrids containing two components or just one type of nanomaterial. It also significantly prevents biofilm formation when coating surfaces, and the AgNP component provides contact bactericidal properties[28,34]. The final relevant study, by Su et al.,[45] also tested the antibacterial properties of a similar hybrid against *S. aureus* and *E. coli* and discovered a significantly enhanced antibacterial performance compared to chitosan alone from the results of diffusion disc tests. The mechanism of action is suggested to be the creation of reactive oxygen species, causing destabilisation of membrane potential, depletion of intracellular adenosine triphosphate, and cell death. Drugs can also be loaded into these hybrids, and a satisfactory response towards heat and cold cycles simulating photothermal therapy conditions is also shown[45].

A schematic of this composite's possible mechanisms of action can be seen in the image above (see Figure 2)[23,35,45,55,60-63,67,70,74,77]. While no pharmacological survey has been done on this hybrid, all three components: silver[12,49,56-57,62], GO[70], and chitosan[78-79], are administrable topically and through the systemic circulation without any toxicity. The AgNP-GO composite has proven safe for human use[81], showing that similar results can be obtained for the new hybrid. However, an in-depth study

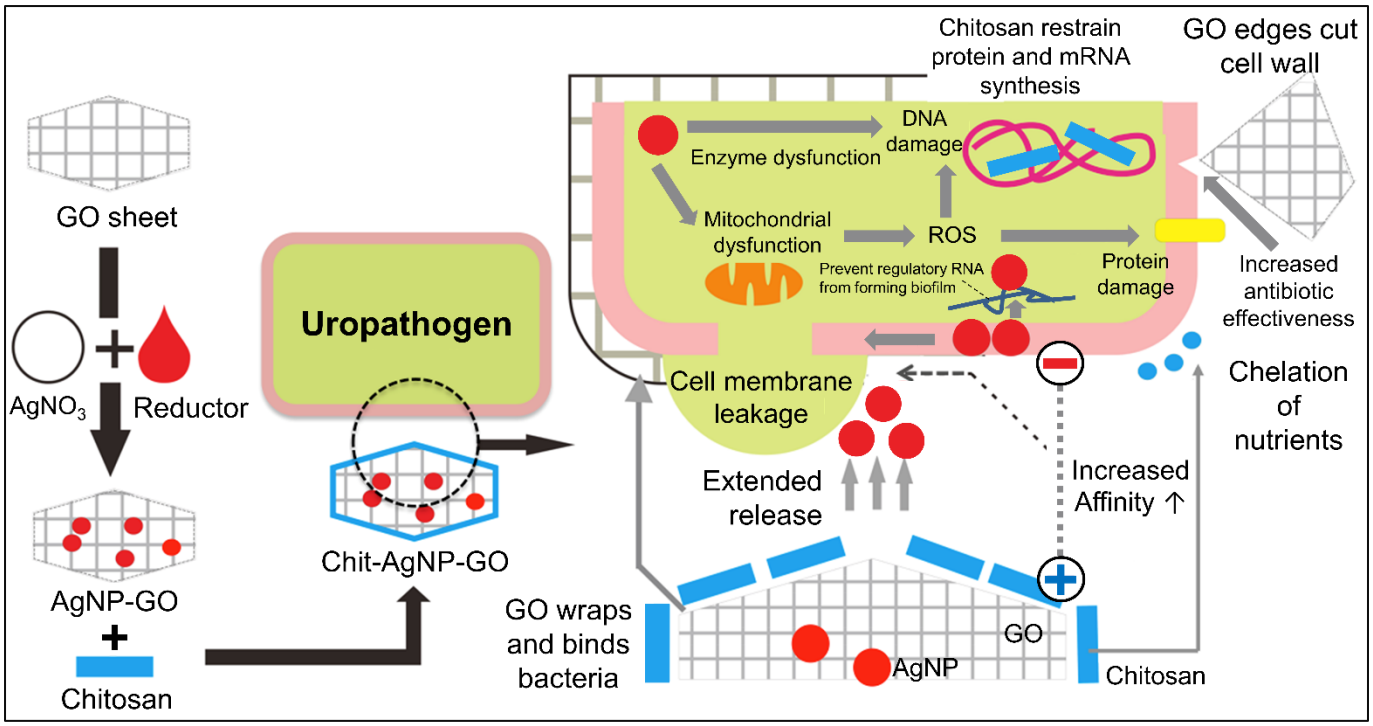

Figure 2. Proposed schematic illustration of the various mechanisms of actions of the Chit-AgNP-GO hybrid as an antimicrobial against uropathogens. Principles of antibacterial mechanisms in this composite include increased affinity to bacterial cell walls, destruction of the cell membrane and cell wall causing cytoplasmic leakage and increased antibiotic effectiveness, oxidative stress, chelation of essential nutrients, destruction of respiration enzymes and DNA, interaction with regulatory mRNA to prevent biofilm creation, and disruption of mRNA and protein synthesis and functions[17,32,49,55,60-63,67,71,75,78].
Abbreviation: AgNP (silver nanoparticle); $AgNO_3$ (silver nitrate); Chit (chitosan); DNA (deoxyribonucleic acid); GO (graphene oxide); ROS (reactive oxygen species).

regarding the pharmacology of this hybrid will be vital in determining whether this new antimicrobial agent is fit for use in the increasingly uphill battle against resistant uropathogens.

3. CONCLUSION

In general, Chit-AgNP-GO is an innovative combination of three antimicrobial components into a hybrid material capable of killing bacteria with a synergistic mechanism of action. Its small size, high density, monodispersion, and various properties outlined in this review are significant advantages in dealing with resistant bacteria. It also eradicates MRSA, a significant superbug with decent MIC and MBC. Individual components of these hybrids also have an extensive record of good preclinical evidence to support their usage as antibacterials. The increasing availability of nanotechnology synthesis and the efficient costs of the three stand-alone ingredients push Chit-AgNP-GO to be an emerging antimicrobial agent with decent feasibility to eliminate resistant uropathogens.

Several limitations in this review include a limitation in the number of studies on the subject in particular, including studies using a combination of Ag^+ ions, GO, and chitosan (either individually or in combination, without other ingredients), studies using these ingredients against only uropathogens, and clinical studies on human patients, both in the field of pharmacokinetics, and pharmacodynamics and efficacy. The heterogeneous results also made it difficult to make general conclusions, and this inconclusive position might warrant a systematic review and meta-analysis in the future. The preclinical evidence for Chit-AgNP-GO as hybrids or as individual components should be developed further from the bench to the bedside. Possible future research areas include *in vivo* studies, integration into simple medical devices (i.e., Foley catheters), and expansion to possible use as systemic antimicrobials. Further studies on the pharmacokinetics/pharmacodynamics of these new nanomedicines must also be done to understand the safety profile of these new compounds.

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Author contributions

MH was the principal investigator of this study, conceptualised the study, and controlled the decision to publish. MH confirmed the authenticity of all data and accepted full responsibility for the overall content of the work. MH, SS, and HA designed the methodology, provided the resources, collected the data, cleaned the data, validated all data analyses, contributed to the formal examination, performed the literature investigation, and drafted the manuscript. MH and HA designed the methodology. MH and SS performed project administration. MH was responsible for software utilisation and visualisation of research findings and supervised the study process thoroughly. All authors critically revised the manuscript and gave final approval for publishing the article.

Data Availability Statements:

All relevant data are available through searches on online repositories of scientific literature

REFERENCES

1. Jameson JL, Loscalzo JJ. Harrison's Nephrology and Acid-Base Disorders. 2nd ed. New York: McGrew Hill Education; 2013. p. 254-64.
2. Öztürk R, Murt A. Epidemiology of urological infections: a global burden. World J Urol. 2020;38(11):2669-79.
3. Linhares I, Raposo T, Rodrigues A, Almeida A. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009). BMC Infect Dis. 2013;13(1):19.
4. Hrbacek J, Cermak P, Zachoval R. Current antibiotic resistance trends of uropathogens in Central Europe: Survey from a tertiary hospital urology department, 2011-2019. Antibiot (Basel). 2020; 9(9):630.
5. Paris D, Caltagirone M, Minzulli P, Valzano A, Ottorino Ferrara FE, Magliano E. Epidemiology and antibiotic resistance in community-acquired lower urinary tract infections in the Milan area. Microbiol Medica. 2020;35(2):26-31.
6. Sugianli AK, Ginting F, Parwati I, de Jong MD, van Leth F, Schultsz C. Antimicrobial resistance among uropathogens in the Asia-Pacific region: a systematic review. JAC-Antimicrobial Resist. 2021;3(1):dlab003.
7. Hadi U, van den Broek P, Kolopaking EP, Zairina N, Gardjito W, Gyssens IC, et al. Cross-sectional study of availability and pharmaceutical quality of antibiotics requested with or without prescription (over the counter) in Surabaya, Indonesia. BMC Infect Dis. 2010;10:203.
8. Wagenlehner FM, Pilatz A, Naber KG, Weidner W. Therapeutic challenges of urosepsis. Eur J Clin Invest. 2008;38 Suppl 2:45-9.
9. Samirah, Darwati, Windarwati, Hardjoeno. Bacterial patterns and its sensitivity in patients suffering from urinary tract infection [in Indonesian]. Indones J Clin Pathol Med Lab. 2006;12(8): 110-3.
10. Awasthi TR, Dutt Pant N, Dahal PR, Pant ND, Dahal PR. Prevalence of multidrug resistant bacteria in causing community acquired urinary tract infection among the patients attending outpatient department of Seti Zonal Hospital, Dhangadi, Nepal. Nepal J Biotechnol. 2015;3(1):55-9.
11. Park YS. Renal scar formation after urinary tract infection in children. Korean J Pediatr. 2012;55(10):367-70.
12. Lansdown ABG. Silver in health care: antimicrobial effects and safety in use. Curr Probl Dermatol. 2006;33:17-34.
13. Loh K, Sivalingam N. Urinary tract infections in pregnancy. Malaysian Fam physician. 2007;2(2):54-7.
14. Marta B, Potara M, Iliut M, Jakab E, Radu T, Imre-Lucaci F, et al. Designing chitosan-silver nanoparticles-graphene oxide nanohybrids with enhanced antibacterial activity against *Staphylococcus aureus*. Colloids Surf A Physicochem Eng Asp. 2015; 487:113-20.
15. Alfuraydi RT, Alminderej FM, Mohamed NA. Evaluation of antimicrobial and anti-biofilm formation activities of novel poly (vinyl alcohol) hydrogels reinforced with crosslinked chitosan and silver nanoparticles. Polymers (Basel). 2022;14(8):1619.
16. Almamad MF, Aldujaili NH. Characterisation of graphene oxide reduced by *Bacillus clausii* and its activity against MDR uropathogenic isolates. IOP Conf Ser Earth Environ Sci. 2021;790(1): 012044.
17. Alshehri SM, Aldalbahi A, Al-hajji AB, Ahmad A, Alhokbany N, Ahamad T, et al. Development of carboxymethyl cellulose-based hydrogel and nanosilver composite as antimicrobial agents for UTI pathogens. Carbohydr Polym. 2016;138:229-36.
18. Alzubaidy FH, Aldujaili NH. Antimicrobial and environmental activity of biogenic CS-GO nanoparticles on uropathogens. IOP Conf Ser Earth Environ Sci. 2022;1029(1):012002.
19. Ashmore D, Chaudhari A, Barlow B, Barlow B, Harper T, Vig K, et al. Evaluation of *E. coli* inhibition by plain and polymer-coated silver nanoparticles. Rev Inst Med Trop Sao Paulo. 2018;60:e18.
20. Campana R, Casettari L, Ciandrini E, Illum L, Baffone W. Chitosans inhibit the growth and the adhesion of *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates on urinary catheters. Int J Antimicrob Agents. 2017;50(2):135-41.
21. Chen J, Peng H, Wang X, Shao F, Yuan Z, Han H. Graphene oxide exhibits broad-spectrum antimicrobial activity against bacterial phytopathogens and fungal conidia by intertwining and membrane perturbation. Nanoscale. 2014;6(3):1879-89.
22. Cobos M, De-La-Pinta I, Quindós G, Fernández MJ, Fernández MD. Graphene oxide-silver nanoparticle nanohybrids: Synthesis, characterisation, and antimicrobial properties. Nanomaterials. 2020;10(2):376.
23. Costa EM, Silva S, Tavaria FK, Pintado MM. Insights into chitosan antibiofilm activity against methicillin-resistant *Staphylococcus aureus*. J Appl Microbiol. 2017;122(6):1547-57.
24. Dayyoub E, Frant M, Reddy S, Liefeith K. Antibacterial and anti-encrustation biodegradable polymer coating for urinary catheter. Int J Pharm. 2017;531(1):205-14.
25. de Faria AF, Martinez DST, Meira SM, de Moraes AC, Brandelli A, Filho AG, et al. Anti-adhesion and antibacterial activity of silver nanoparticles supported on graphene oxide sheets. Colloids Surf B Biointerfaces. 2014;113:115-24.
26. de Saravia SG, Rastelli SE, Angulo-Pineda C, Palza H, Viera MR. Anti-adhesion and antibacterial activity of silver nanoparticles and graphene oxide-silver nanoparticle composites. Matéria (Rio J). 2020;25(2):e12771.
27. Dragland IS, Rukke HV, Stenhagen ISR, Lönn-Stensrud J, Kopperud HM. Antibacterial and antibiofilm effect of low viscosity chitosan against *Staphylococcus epidermidis*. Int J Microbiol. 2016;2016:9159761.
28. Khawaja H, Zahir E, Asghar MA, Asghar MA. Graphene oxide, chitosan and silver nanocomposite as a highly effective antibacterial agent against pathogenic strains. Colloids Surfaces A Physicochem Eng Asp. 2018;555:246-55.
29. Venkat Kumar G, Su CH, Velusamy P. Surface immobilisation of kanamycin-chitosan nanoparticles on polyurethane ureteral stents to prevent bacterial adhesion. Biofouling. 2016;32(8):861-70.
30. Lopez-Carrizales M, Velasco KI, Castillo C, Flores A, Magaña M, Martinez-Castanon GA, et al. *In-vitro* synergism of silver nanoparticles with antibiotics as an alternative treatment in multiresistant uropathogens. Antibiotics. 2018;7(2):50.
31. Maruthupandy M, Govindan R, Shine K, Thangasamy V, Alharbi NS, Thillaichidambaram M, et al. Anti-biofilm investigation of graphene/chitosan nanocomposites against biofilm-producing *P. aeruginosa* and *K. pneumoniae*. Carbohydr Polym. 2020;230: 115646.

32. de Moraes ACM, Lima BA, de Faria AF, Brocchi M, Alves OL, Moraes ACM de, et al. Graphene oxide-silver nanocomposite as a promising biocidal agent against methicillin-resistant *Staphylococcus aureus*. Int J Nanomedicine. 2015;10:6847-61.
33. Paul S, Seema NP. *In vitro* antibacterial potential of chitosan and its derivatives on pathogenic Enterobacteriaceae. Natl J Physiol Pharm Pharmacol. 2015;5(2):1-6.
34. Pounraj S, Somu P, Paul S. Chitosan and graphene oxide hybrid nanocomposite film doped with silver nanoparticles efficiently prevents biofouling. Appl Surf Sci. 2018;452:487-97.
35. Rahuman HBH, Dhandapani R, Palanivel V, Thangavelu S, Paramasivam R, Muthupandian S. Bioengineered phytomolecules-capped silver nanoparticles using *Carissa carandas* leaf extract to embed on to urinary catheter to combat UTI pathogens. PLoS One. 2021;16(9):e0256748.
36. Rajivgandhi G, Maruthupandy M, Veeramani T, Quero F, Li WJ. Anti-ESBL investigation of chitosan/silver nanocomposites against carbapenem-resistant *Pseudomonas aeruginosa*. Int J Biol Macromol. 2019;132:1221-34.
37. Rajivgandhi G, Maruthupandy M, Muneeswaran T, Anand M, Quero F, Manoharan N, et al. Biosynthesised silver nanoparticles for inhibition of antibacterial resistance and biofilm formation of methicillin-resistant coagulase negative staphylococci. Bioorg Chem. 2019;89:103008.
38. Rodríguez-Serrano C, Guzmán-Moreno J, Ángeles-Chávez C, Rodríguez-González V, Ortega-Sigala JJ, Ramírez-Santoyo RM, et al. Biosynthesis of silver nanoparticles by *Fusarium scirpi* and its potential as antimicrobial agent against uropathogenic *Escherichia coli* biofilms. PLoS One. 2020;15(3):e0230275.
39. Rubini D, Banu SF, Subramani P, Hari BNV, Gowrishankar S, Pandian SK, et al. Extracted chitosan disrupts quorum sensing mediated virulence factors in urinary tract infection-causing pathogens. Pathog Dis. 2019;77(1):1-13.
40. Rubini D, Vishnu P, Jayasankari S, Vedahari BN, Nithyanand P. Suppressing the phenotypic virulence factors of uropathogenic *Escherichia coli* using marine polysaccharide. Microb Pathog. 2020;141:103973.
41. Sajjad S, Uzair B, Shaukat A, Jamshed M, Leghari SAK, Ismail M, et al. Synergistic evaluation of AgO2 nanoparticles with ceftriaxone against CTXM and blaSHV genes-positive ESBL-producing clinical strains of uropathogenic *E. coli*. IET Nanobiotechnol. 2019;13(4):435-40.
42. Samanta S, Banerjee J, Das B, Mandal J, Chatterjee S, Ali KM, et al. Antibacterial potency of cytocompatible chitosan-decorated biogenic silver nanoparticles and molecular insights towards cell-particle interaction. Int J Biol Macromol. 2022;219:919-39.
43. Shafreen RB, Seema S, Ahamed AP, Thajuddin N, Ali Alharbi S. Inhibitory effect of biosynthesised silver nanoparticles from extract of *Nitzschia palea* against Curli-mediated biofilm of *Escherichia coli*. Appl Biochem Biotechnol. 2017;183(4):1351-61.
44. Srinivasan R, Vigneshwari L, Rajavel T, Durgadevi R, Kannappan A, Balamurugan K, et al. Biogenic synthesis of silver nanoparticles using *Piper betle* aqueous extract and evaluation of its anti-quorum sensing and antibiofilm potential against uropathogens with cytotoxic effects: an *in vitro* and *in vivo* approach. Environ Sci Pollut Res. 2018;25(11):10538-54.
45. Su Z, Sun D, Zhang L, He M, Jiang Y, Millar B, et al. Chitosan/silver nanoparticle/graphene oxide nanocomposites with multi-drug release, antimicrobial, and photothermal conversion functions. Materials (Basel). 2021;14(9):2351.
46. Blango MG, Ott EM, Erman A, Veranic P, Mulvey MA. Forced resurgence and targeting of intracellular uropathogenic *Escherichia coli* reservoirs. PLoS One. 2014;9(3):e93327.
47. Erman A, Hergouth VK, Blango MG, Kos MK, Mulvey MA, Veranic P. Repeated treatments with chitosan in combination with antibiotics completely eradicate uropathogenic *Escherichia coli* from infected mouse urinary bladders. J Infect Dis. 2017; 216(3):375-81.
48. Mala R, Aglin AA, Selvaraj A, Celsia R, Geerthika S, Vazagapriya C, et al. Foley catheters functionalised with a synergistic combination of antibiotics and silver nanoparticles resist biofilm formation. IET Nanobiotechnol. 2017;11:612-20.
49. Lederer JW, Jarvis WR, Thomas L, Ritter J. Multicenter cohort study to assess the impact of a silver-alloy and hydrogel-coated urinary catheter on symptomatic catheter-associated urinary tract infections. J Wound Ostomy Continence Nurs. 2014;41(5):473-80.
50. Mijnendonckx K, Leys N, Mahillon J, Silver S, Van Houdt R. Antimicrobial silver: uses, toxicity and potential for resistance. BioMetals. 2013;26(4):609-21.
51. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. J Biomed Mater Res. 2000;52 (4):662-8.
52. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. Appl Environ Microbiol. 2008;74(7):2171-8.
53. Holt KB, Bard AJ. Interaction of silver(I) ions with the respiratory chain of *Escherichia coli*: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag+. Biochemistry. 2005;44(39):13214-23.
54. Bovenkamp GL, Zanzen U, Krishna KS, Hormes J, Prange A. X-ray absorption near-edge structure (XANES) spectroscopy study of the interaction of silver ions with *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. Appl Environ Microbiol. 2013;79(20):6385-90.
55. Xu FF, Imlay JA. Silver(I), mercury(II), cadmium(II), and zinc(II) target exposed enzymic iron-sulfur clusters when they toxify *Escherichia coli*. Appl Environ Microbiol. 2012;78(10):3614-21.
56. Saint S, Elmore JG, Sullivan SD, Emerson SS, Koepsell TD. The efficacy of silver alloy-coated urinary catheters in preventing urinary tract infection: a meta-analysis. Am J Med. 1998;105(3): 236-41.
57. Johnson JR, Kuskowski MA, Wilt TJ. Systematic review: Antimicrobial urinary catheters to prevent catheter-associated urinary tract infection in hospitalised patients. Ann Intern Med. 2006; 144(2):116-26.
58. Raftery TD, Kerscher P, Hart AE, Saville SL, Qi B, Kitchens CL, et al. Discrete nanoparticles induce loss of *Legionella pneumophila* biofilms from surfaces. Nanotoxicology. 2014;8(5):477-84.
59. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, et al. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J Proteome Res. 2006;5(4):916-24.
60. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology. 2007;(22):225103.
61. Chen M, Yang Z, Wu H, Pan X, Xie X, Wu C. Antimicrobial activity and the mechanism of silver nanoparticle thermosensitive gel. Int J Nanomedicine. 2011;6:2873-7.
62. Lansdown ABG. A pharmacological and toxicological profile of silver as an antimicrobial agent in medical devices. Adv Pharmacol Sci. 2010;2010:910686.
63. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod. 2014;40(2):285-90.
64. Kalishwaralal K, BarathManiKanth S, Pandian SRK, Deepak V, Gurunathan S. Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Colloids Surfaces B. 2010;79(2):340-4.
65. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano. 2009;3(2):279-90.
66. Dziendzikowska K, Gromadzka-Ostrowska J, Lankoff A, Oczkowski M, Krawczyńska A, Chwastowska J, et al. Time-dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J Appl Toxicol. 2012;32(11):920-8.
67. Liu S, Zeng TH, Hofmann M, Burcombe E, Wei J, Jiang R, et al. Antibacterial activity of graphite, graphite oxide, graphene oxide,

and reduced graphene oxide: membrane and oxidative stress. ACS Nano. 2011;5(9):6971-80.
68. Perreault F, de Faria AF, Nejati S, Elimelech M. Antimicrobial properties of graphene oxide nanosheets: Why size matters. ACS Nano. 2015;9(7):7226-36.
69. Chen Y, Wu W, Xu Z, Jiang C, Han S, Ruan J, et al. Photothermal-assisted antibacterial application of graphene oxide-Ag nanocomposites against clinically isolated multi-drug resistant *Escherichia coli*. R Soc Open Sci. 2020;7(7):192019.
70. Chang Y, Yang ST, Liu JH, Dong E, Wang Y, Cao A, et al. *In vitro* toxicity evaluation of graphene oxide on A549 cells. Toxicol Lett. 2011;200(3):201-10.
71. Yang K, Wan J, Zhang S, Zhang Y, Lee ST, Liu Z. *In vivo* pharmacokinetics, long-term biodistribution, and toxicology of PEGylated graphene in mice. ACS Nano. 2011;5(1):516-22.
72. Dutta J, Dutta PP. Antimicrobial activity of chitin, chitosan, and their oligosaccharides. In: Kim SK, editor. Chitin, chitosan, Oligosaccharides and their derivatives biological activities and Applications. 1st ed. Boca Raton: Taylor and Francis Group; 2010. p. 195-214.
73. Aranaz I, Alcántara AR, Civera MC, Arias C, Elorza B, Heras Caballero A, et al. Chitosan: An overview of its properties and applications. Polymers (Basel). 2021;13(19):3256.
74. Potara M, Jakab E, Damert A, Popescu O, Canpean V, Astilean S. Synergistic antibacterial activity of chitosan-silver nanocomposites on *Staphylococcus aureus*. Nanotechnology. 2011;22(13): 135101.
75. Piras AM, Maisetta G, Sandreschi S, Gazzarri M, Bartoli C, Grassi L, et al. Chitosan nanoparticles loaded with the antimicrobial peptide temporin B exert a long-term antibacterial activity *in vitro* against clinical isolates of *Staphylococcus epidermidis*. Front Microbiol. 2015;6:372.
76. Ong TH, Chitra E, Ramamurthy S, Siddalingam RP, Yuen KH, Ambu SP, et al. Chitosan-propolis nanoparticle formulation demonstrates antibacterial activity against *Enterococcus faecalis* biofilms. PLoS One. 2017;12(3):e0174888.
77. Ibrahim HM, El-Bisi MK, Taha G, El-Alfy E. Chitosan nanoparticles loaded antibiotics as drug delivery biomaterial. J App Pharm Sci. 2015;5(10):85-90.
78. Mukhopadhyay P, Bhattacharya S, Nandy A, Bhattacharyya A, Mishra R, Kundu PP, et al. Assessment of *in vivo* chronic toxicity of chitosan and its derivates used as oral insulin carriers. Toxicol Res. 2015;4(2):289-90.
79. Hirano S, Seino H, Akiyama Y, Nonaka I. Chitosan: A Biocompatible Material for Oral and Intravenous Administrations. In: Gebelein CG, Dunn RL, editors. Progress in Biomedical Polymers. Boston, MA: Springer US; 1990. p. 283-90.
80. Teodoro JS, Gomes AP, Varela AT, Duarte FV, Rolo AP, Palmeira CM. Hepatic and skeletal muscle mitochondrial toxicity of chitosan oligosaccharides of normal and diabetic rats. Toxicol Mech Methods. 2016;26(9):650-7.
81. Lima DDS, Gullon B, Cardelle-Cobas A, Brito LM, Rodrigues KA, Quelemes PV, et al. Chitosan-based silver nanoparticles: A study of the antibacterial, antileishmanial and cytotoxic effects. J Bioact Compat Polym. 2017;32(4):397-410.