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## Carbon-based two-dimensional (2D) materials: a next generation biocidal agent

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Two-dimensional materials (2D-Ms) such as graphene, carbon nitride ( $C_3N_4$ ), and MXene have attracted significant attention due to their excellent physico-chemical properties, including high surface area-to-volume ratio, biocompatibility, mechanical strength, high conductivity, etc. There has been growing interest in utilizing 2D-Ms for antibacterial applications including photo-antibacterial activity. The rise of antibiotic-resistant bacteria has made new antibiotic materials imperative, and 2D-Ms have shown promise in this area. One of the main advantages of 2D-Ms for antibacterial applications is their high surface area-to-volume ratio, which increases contact between the material and bacteria, leading to more effective antibacterial properties. Additionally, some carbon-based 2D-Ms (CB-2D-Ms) have been shown to have intrinsic antibacterial properties, such as graphene and its derivatives,  $g-C_3N_4$ , MXene, etc., as backbone carbon provides mechanical support, which can be further enhanced by functionalization with biocidal agents (metals/metal oxides, surface functional groups, and polymers). This mini-review highlights the latest developments in CB-2D-Ms, such as graphene and its derivatives,  $C_3N_4$ , MXenes, etc., as antibiotic materials to control bacterial infection. Herein, we correlate the exclusive range of 2D properties of CB-2D-Ms with their antimicrobial actions. Lastly, challenges and future perspectives in this area of CB-2D-Ms are also described.

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### 1. Introduction

Bacterial infectious disease is one of the major threats to human society, as various mutations cause the development of hazardous bacteria and viruses, which is of significant concern. Infections grown from these bacteria and viruses are bringing serious threats to the human population globally, causing an increased mortality rate. Numerous antibiotics are available on the market to treat infection from available bacteria and viruses. As these antibiotics are widely used, antibacterial resistance has been developed for these available antibiotics, reducing the efficacy of these antibiotics.<sup>1–5</sup> In this context, the discovery and development of novel antibiotic materials are required to fulfil the growing population's demand.

Numerous antibiotics have been developed so far that effectively kill/inhibit bacterial strains. The excessive use of these antibiotics might lead to the development of resistance against these antibiotics that significantly enhance mortality

and morbidity.<sup>6–10</sup> Therefore, there is a need to develop newer antibiotic materials or modify existing antibiotics so as to inhibit the development of resistance against these drugs. In this aspect, nanomaterials (NMs), mainly metal oxides, metal hydroxides, and metal-doped carbon materials, might be able to control bacterial infection without the development of bacterial resistance.<sup>11–13</sup>

Two-dimensional materials (2D-Ms) have attracted the scientific community's attention because of their large pore size distribution, high surface area, excellent mechanical properties, and high chemical resistance. 2D-Ms are in demand because of their novel characteristics like easy tunability by incorporation of surface functional groups, metals/metal oxides and polymers, which significantly improve their applicability towards end applications including biocidal agents with minimal toxicity or high biocompatibility. Some widely used 2D-Ms are graphene, graphene oxide (GO), a reduced form of graphene oxide (r-GO),  $C_3N_4$  nanosheets, boron nitride (BN) nanosheets, MXene, TMDs etc. Among them, CB-2D-Ms, like graphene, GO, rGO, MXene, and  $C_3N_4$ , are of especial interest due to their large mechanical strength. CB-2D-Ms can provide mechanical flexibility together with large surface area and pore size distribution.<sup>14–25</sup> Additionally, several studies have shown that CB-2D-Ms can effectively inhibit the growth of various bacteria, including *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). Usually, their antibacterial activity

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can be attributed to various mechanisms, such as disruption of bacterial cell membranes, oxidative stress, and inhibition of bacterial adhesion. In addition to their antibacterial properties, CB-2D-Ms have other advantages, such as biocompatibility, mechanical flexibility, and ease of functionalization.<sup>26–29</sup> These properties make CB-2D-Ms promising candidates for killing/inhibiting various bacterial strains, with prospects for effective use in different applications such as wound healing, medical implants, and water purification. Simultaneously, they are biocompatible and have shown a wide range of antibiotic properties, making them excellent candidates for use as antibacterial agents. CB-2D-Ms are in demand as antibacterial agents as they can provide good support as a substrate due to their tunable surface, and can be easily functionalized and changed for various applications. However, research is still required to change the surface properties for antibacterial applications.<sup>30–37</sup> In general, there are plenty of advantages that suggest to use of CB-2D-Ms as biocidal agents, mainly high mechanical stability, high biocompatibility, easy functionalization/tunability, and mechanical flexibility. Fig. 1 shows a schematic illustration of CB-2D-Ms and their biocidal activity. This mini-review covers the recent developments in the field of CB-2D-Ms for antibacterial application. This article also highlights the newer strategies to modify the surface properties of these CB-2D-Ms. We also highlight and discuss the various methods adopted to improve the efficiency of these materials.

## 2. Biocidal activity of CB-2D-Ms

CB-2D-Ms are used in several applications, including wastewater treatment, agriculture, energy storage, biomedicine *etc.* Moreover, applications of CB-2D-Ms as antibacterial agents are one of the growing fields that need to be explored, as most bacterial strains develop resistance against antibiotic drugs.<sup>25,38–40</sup> CB-2D-Ms, like graphene, rGO, MXene, and  $\text{C}_3\text{N}_4$ , can efficiently be used as antibiotic materials without development of bacterial resistance with minimal toxicity.

### 2.1. Graphene and its derivatives

Graphene is one of the most in-demand materials in wastewater treatment and energy storage applications. Furthermore, graphene derivatives such as GO and rGO are gaining widespread attention as antibacterial agents. However, antibacterial

properties demand surface functionality by chemicals, metals/metal oxides, and polymers.<sup>41–44</sup> Several researches show different functionalization strategies to increase the antibacterial properties of graphene and its derivatives. For instance, Khanam *et al.* synthesized GO using a modified Hummers' method and then synthesized r-GO using *Allium cepa* extract as reducing agent. This biosynthesized r-GO was applied as an antimicrobial agent against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. faecalis* and *S. aureus*) bacterial strains. The data suggested that the r-GO significantly reduces the cell viability and its efficacy depends on the incubation time.<sup>45</sup> Another study by Bykkam synthesized ZnO nanoparticles (NPs), loaded a few layers of graphene and applied for antibacterial use. Bykkam found that the synthesized ZnO-loaded few-layer graphene nanocomposite proved to be an excellent antibacterial agent against *E. coli* and *S. typhi*.<sup>46</sup> Ali *et al.* synthesized graphene incorporated chitosan/gelatin nanofibers using electrospinning process for antibacterial and wound healing applications. The data indicate that the prepared nanofibrous mat effectively inhibits/kills *E. coli* and *S. aureus*. Moreover, migration of cells significantly improved upon incorporation of graphene, thereby healing wounds.<sup>47</sup> Jiang *et al.* synthesized Ag-loaded graphene, which exhibits excellent antibacterial properties. The authors decorated 40–50 nm Ag-NPs on graphene layers to achieve maximum performance towards bacterial degradation.<sup>48</sup> Another study by Krishnamoorthy *et al.* synthesized graphene nanolayers using the liquid-phase exfoliation method. The exfoliated graphene proved to be an excellent candidate against *E. coli* and *S. typhimurium*. The study also mentioned the mechanistic approach of the antibacterial nature of graphene synthesized using liquid-phase exfoliation as free radicals are involved in the antimicrobial activity, especially reactive oxygen species (ROS), which react with the cell wall and leading to bacterial cell death.<sup>49</sup>

Derivatives of graphene, especially GO, also proved to be excellent antibacterial agents due to their properties like electrical conductivity, high surface area and pore size distribution, excellent physico-chemical properties and extraordinary mechanical strength with great functionality. Several literature studies claim that GO's antibacterial activity lies in its ability to generate ROS, which directly attack bacterial cell walls and leads to cell death.<sup>50</sup> GO demonstrates extraordinary properties, including mild cytotoxicity, and a simple synthesis process for large-scale production with economic viability. Another advantage is that bacterial killing involves two mechanisms, physical destruction and chemical oxidation, which reduces bacterial resistance.<sup>51</sup> Several literature studies have proved the efficiency of GO and its composites as antibacterial agents, including that of Shamsi *et al.* who synthesized gallic acid-loaded GO and used the synthesized nanocomposites as an antibacterial agent against *S. aureus*. The authors evaluated the antibacterial activity using the disc-diffusion method (MRSA and methicillin-sensitive SA (MSSA)) at a fixed dose of gallic acid-modified GO. The authors observed that the antibacterial activity of gallic acid-modified GO against MRSA increased significantly at lower concentrations and proved that the synthesized nanocomposite of gallic acid-modified GO is effective against

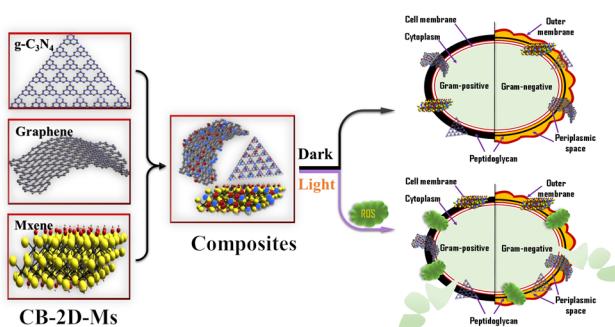


Fig. 1 A schematic illustration of CB-2D-Ms and their biocidal activity.



multi-drug-resistant bacteria.<sup>52</sup> Li *et al.* synthesized CuO-NPs-loaded GO nanosheets and applied them as an antibacterial agent against *P. syringae* *pv. tomato*. The authors observed that synthesized CuO-NPs-loaded GO-based nanocomposite has high efficiency in acting as a biocide and has great potential for managing crop diseases.<sup>53</sup> Ping Li *et al.* functionalized GO with guanidine polymer and applied it as an antibacterial agent against both Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*) and found the efficiency of functionalized GO as a potential antibacterial agent.<sup>54</sup> Another study by Jaworski *et al.* decorated GO with Ag-NPs using an ultrasonic method and tested it against Gram-positive bacteria (*S. aureus* and *S. epidermidis*), Gram-negative bacteria (*E. coli*), and pathogenic fungi (*Candida albicans* (*C. albicans*)). The authors reported the efficiency of functionalized GO nanocomposite as an antibacterial agent.<sup>55</sup> Tang *et al.* again synthesized the same nanocomposite and studied the mechanism of the synthesized nanocomposite for bacterial degradation. The authors mentioned that the synthesized composite acts as an antibacterial agent and generates ROS to degrade bacterial cell walls.<sup>56</sup> Another graphene derivative is r-GO, which can be synthesized using a simple reduction process of GO, as GO consists of several functional groups, which somehow impact the dispersion ability of GO. To overcome this issue, GO undergoes a reduction process, which subsequently improves the dispersion as well as antibacterial performance.

Several studies highlighted the synthesis of r-GO and its composites and applied them as antibacterial agents. For example, a nanocomposite of r-GO and Ag-NPs was synthesized and applied for dual application (1) as an antibacterial and (ii) as a cancer biomarker sensor.<sup>57</sup> Fig. 2 shows the SEM images of (a) *E. coli*, (b) exposed *E. coli*, (c) *S. aureus*, and (d) exposed *S. aureus*. The SEM images clearly indicate the killing/inhibition of bacterial strains.

Another study by Moghayedi *et al.* synthesized silver NPs doped with phosphomolybdate-modified rGO nanocomposite and studied the kinetics and mechanism of bacterial degradation.<sup>58</sup> Alsharaeh *et al.* synthesized silver NPs-doped rGO nanocomposites and observed the antibacterial behaviour of the synthesized nanocomposites.<sup>59</sup> The studies mentioned above clearly indicate the applicability of graphene and its derivatives as excellent antibacterial agents for various Gram-positive and Gram-negative bacteria which can demonstrate 100% bacterial degradation/killing/inhibiting ability within a very short time and low dose showing their excellence towards bacterial degradation by generating ROS.

## 2.2. Carbon nitrides and borides

Another class of 2D-M is  $C_3N_4$ , as this is a metal-free class of CB-2D-Ms. The synthesis of  $C_3N_4$  is very simple and easy as it requires only carbon and nitrogen derivatives, mainly compounds of urea and melamine. After polycondensation, a layered structure is formed, assembled through van der Waals interaction. Due to various properties like high chemical inertness and structural stability, these materials are widely used in different applications, including wastewater treatment, solar

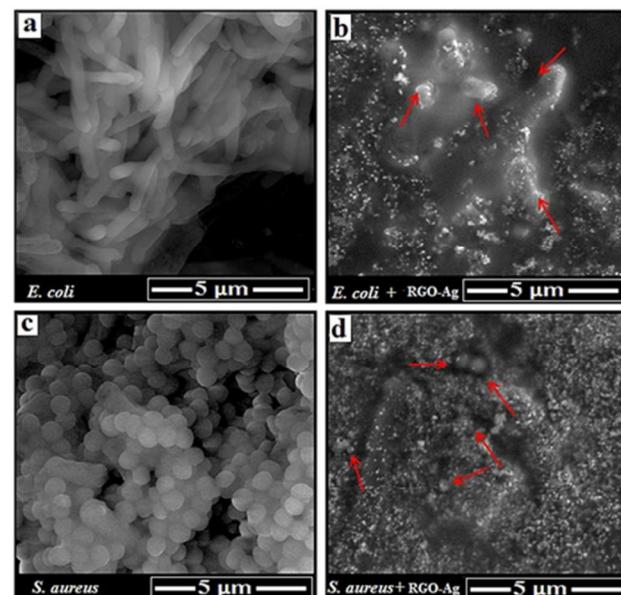


Fig. 2 SEM images of (a) *E. coli*, (b) exposed *E. coli*, (c) *S. aureus*, and (d) exposed *S. aureus*. Reproduced from ref. 57 with permission from RSC, copyright 2016 under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

cells, energy storage, fuel cells, *etc.*<sup>60–62</sup> Moreover, due to low band gap value, these materials are widely considered as semiconductors and are mainly used in photocatalysis. The antibacterial activity of graphitic  $C_3N_4$  ( $g-C_3N_4$ ) has also been studied by several authors and it has been proved that this metal-free carbon nitrogen skeleton could significantly disrupt bacterial cell walls under solar irradiation. Several studies have reported the photo-antibacterial application of  $C_3N_4$ -based semiconductor material. For example, Cui *et al.* synthesized  $g-C_3N_4$  nanosheets. Further, the synthesized  $g-C_3N_4$  nanosheets were subjected to plasma treatment to reduce surface defects and expose nitrogen vacancies. The synthesized  $g-C_3N_4$  was applied as a photo-antibacterial agent, demonstrating excellent performance against Gram-positive and Gram-negative bacteria.<sup>63</sup> Fang *et al.* synthesized phosphorus and sulfur co-doped  $C_3N_4$  nanosheets and applied them for photo-antibacterial use. The synthesized material showed excellent activity towards bacterial cell wall degradation.<sup>64</sup> Sun *et al.* synthesized GO- $g-C_3N_4$  and tested it against an *E. coli* bacterial strain. The data indicate that GO- $g-C_3N_4$  killed 97.9% *E. coli* within 120 min under solar irradiation.<sup>65</sup> Shoran *et al.* synthesized  $CeO_2$ - $g-C_3N_4$ -based composite materials for photodegradation of Gram-negative (*S. abony* and *E. coli*) and Gram-positive (*S. aureus* and *B. cereus*) bacteria. The data indicate that the prepared  $CeO_2$ - $g-C_3N_4$ -based composite materials effectively photodegrade both types of bacterial strains.<sup>66</sup> Wang *et al.* synthesized  $Cu_2O$ ,  $g-C_3N_4$ , and  $Cu_2O$ - $g-C_3N_4$ -based composite materials for photodegradation of bacteria. The data suggested that the prepared  $Cu_2O$ - $g-C_3N_4$ -based composite materials have antibacterial activity, which was significantly improved under solar light irradiation.<sup>67</sup> Yin *et al.* synthesized  $BiFeO_3$ - $g-C_3N_4$  mushroom heterojunction for photo-antibacterial and wound healing applications. The data indicate that the



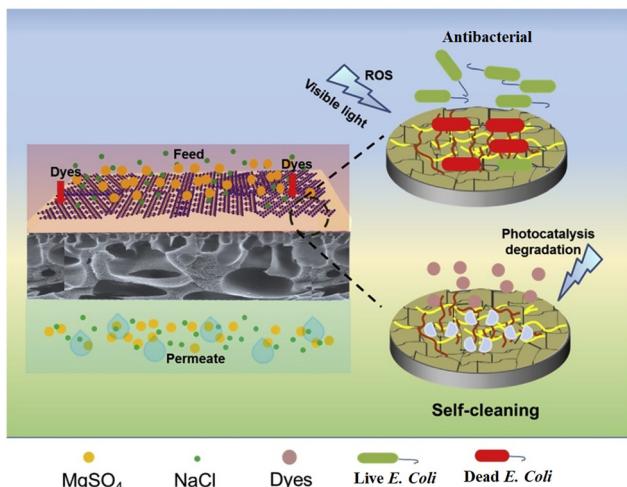


Fig. 3 A schematic illustration of  $\text{g-C}_3\text{N}_4$ -based self-cleaning membranes for the photocatalytic degradation of dyes and bacteria. Reproduced from ref. 69 with permission from Elsevier, copyright 2019.

prepared composite materials inhibit/kill *E. coli* and *S. aureus* and promote healing of wounds.<sup>68</sup> Another study synthesized  $\text{g-C}_3\text{N}_4$ -functionalized self-cleaning membranes for antibacterial applications. The data suggested that the  $\text{g-C}_3\text{N}_4$ -functionalized self-cleaning membranes effectively degrade bacterial strains and dyes, which provide a newer avenue for water treatment applications.<sup>69</sup> This study provides newer opportunities to develop self-cleaning membranes for the removal of contaminants from water. However, further research is required that should be focused on the stability of self-cleaning membranes. Fig. 3 shows a schematic illustration of  $\text{g-C}_3\text{N}_4$ -based functionalized self-cleaning membranes for the photodegradation of dyes and bacteria.

Gao *et al.* synthesized  $\text{Fe}^{3+}$ -doped alkalized  $\text{C}_3\text{N}_4$  and tested it against *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, and *S. aureus* bacterial strains and found it effective against these bacteria.<sup>70</sup> Fig. 4 shows a schematic representation of the photo-antibacterial activity of  $\text{C}_3\text{N}_4$ . These studies support the

antibacterial application of  $\text{C}_3\text{N}_4$ . Under light absorption, due to a large band gap, absorption of light is limited, which limits the overall performance. Moreover, the addition of dopant and organic functionalization can decrease the band gap value and, subsequently, bacterial cell wall degradation. Furthermore,  $\text{g-C}_3\text{N}_4$ -based nanocomposites have been synthesized, which helps expand visible light absorption ranges and enhance their antibacterial performance. However, some concerns remain, such as the generation of toxins during cell wall degradation, which interfere with protein synthesis and oxidize organics. Another concern is that the response of  $\text{C}_3\text{N}_4$  towards bacterial degradation mainly depends on absorption of particular wavelengths. In this context, band gap modulation is required to enhance the absorption in a broad range.

The  $\text{g-C}_3\text{N}_4$  also shows antibacterial activity without exposure to light by incorporating metal/metal oxide NPs. For instance, Qamar *et al.* synthesized  $\text{g-C}_3\text{N}_4\text{-Cr-ZnO}$ -based composite material with antibacterial activity against Gram-negative (*E. coli*) and Gram-positive (*Bacillus subtilis*, *S. aureus*, and *S. salivarius*) bacteria. The data suggested that the prepared composite had excellent antibacterial activity due to synergistic effects of the heterojunction between  $\text{g-C}_3\text{N}_4$  and Cr-ZnO.<sup>71</sup> Another report shows the synthesis of GO- $\text{g-C}_3\text{N}_4$  for photodegradation of amyloid  $\beta$  protein. This study might open newer possibilities to kill bacteria by degradation of bacterial protein.<sup>72</sup> These studies suggested that  $\text{g-C}_3\text{N}_4$  materials have antibacterial activity against various bacterial strains under solar light irradiation as well as without use of light.

### 2.3. MXene (metal carbide)

MXene, another CB-2D-M, has been widely used as an antibacterial agent. MXene is synthesized using the MAX phase (compound of metal aluminium carbide). The general formula of the MAX phase is  $\text{M}_{n+1}\text{X}_n\text{T}_x$ , where M is a transition metal element. This newly discovered material finds significant interest in energy storage due to its large conductivity. However, novel applications include wastewater treatment and solar cells.<sup>73-76</sup> In addition, MXene and its derivatives are now gradually entering the biomedical science and drug delivery fields due to their excellent biocompatibility, great physicochemical-biological properties, contact-killing capacity, phototherapy activity and non-toxic behaviour. As reported, MXene can induce oxidative stress, resulting in cell damage, and can be applied as an antibacterial agent against various pathogens.<sup>77</sup> Several literature studies report the application of MXene as an antibacterial agent. For example, Gao *et al.* synthesized  $\text{Ti}_3\text{C}_2\text{T}_x$  MXene nanosheets and tested them against methicillin-resistant *Staphylococcus aureus*.<sup>77</sup> Another study by Tahir *et al.* synthesized  $\text{Gd}^{3+}$ -doped vanadium oxide-based 2D-MXene nanosheet composite using the sol-gel method and applied it against Gram-positive *S. aureus* and Gram-negative *P. vulgaris* strains of bacteria.<sup>78</sup> Shamsabadi *et al.* synthesized colloidal  $\text{Ti}_3\text{C}_2\text{T}_x$  MXene nanosheets having lateral sizes of 0.09, 0.35, 0.57, and 4.40  $\mu\text{m}$  and tested them against *E. coli* and *B. subtilis* bacteria.<sup>17</sup> Rasool *et al.* synthesized an MXene nanosheet-based antibacterial membrane on polyvinylidene fluoride (PVDF) support, tested it against *E. coli* and *B. subtilis*,

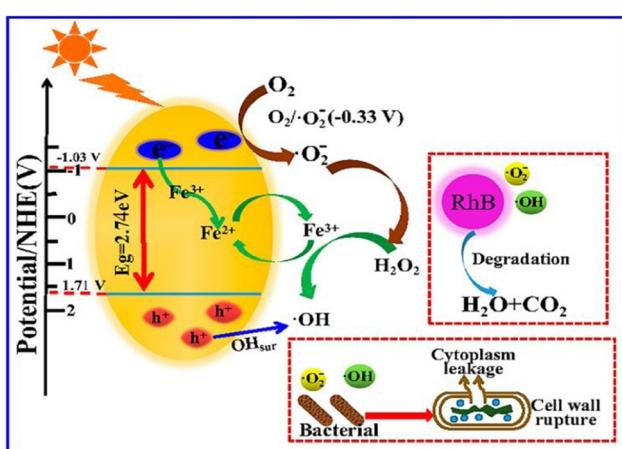


Fig. 4 Schematic representation of photo-antibacterial activity. Reproduced from ref. 70 with permission from MDPI, copyright 2020 under Creative Commons Attribution 4.0 International License (CC BY 4.0).



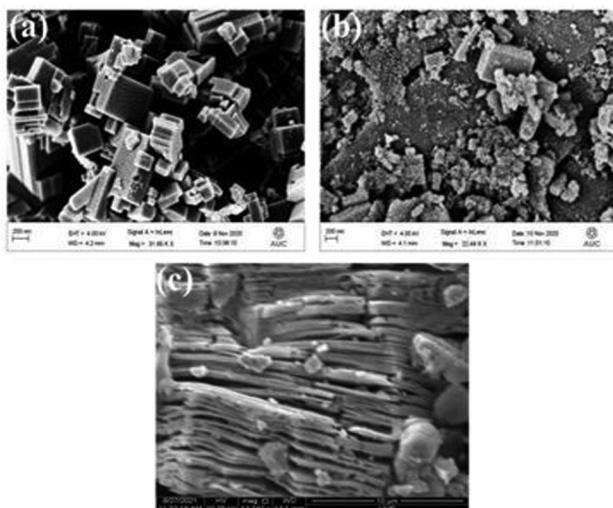


Fig. 5 SEM images of (a)  $\text{WO}_3$ , (b)  $\text{WO}_3$ -MXene, and (c) MXene. Reproduced from ref. 80 with permission from MDPI, copyright 2022 under a Creative Commons Attribution 4.0 International License (CC BY 4.0).

and found 99% growth inhibition of both bacteria over the membrane surface.<sup>79</sup> Warsi *et al.* synthesized an MXene/ $\text{WO}_3$  nanosheet composite using a simple sonication method. The authors observed that the synthesized nanocomposite showed good antibacterial properties against Gram-positive bacterial strains; however, cell damage of Gram-negative strains is concentration-dependent.<sup>80</sup> Fig. 5. shows SEM images of (a)  $\text{WO}_3$ , (b)  $\text{WO}_3$ -MXene, and (c) MXene. The interlayer spacing between two adjacent layers confirms the formation of MXene. Talreja *et al.* synthesized Cu-MXene to control bacterial infection effectively.<sup>81</sup> These studies proved the great potential of MXene and MXene-based nanocomposites as antibacterial agents. The MXene nanosheets were found to damage bacterial cells significantly quickly, releasing bacterial DNA followed by bacterial cell dispersion, resulting in bacterial cell death. In conclusion, MXene and its composites are promising antibacterial agents against various pathogens.

Table 1 summarizes the different CB-2D-Ms and their anti-bacterial activities. The data suggested that the CB-2D-Ms effectively inhibited/killed bacterial strains. The antibacterial activity is mainly due to the metals incorporated within the CB-2D-Ms.

### 3. Mechanism of action

The mechanism of killing or inhibiting bacteria is quite different for CB-2D-Ms due to their unique physical and chemical properties. CB-2D-Ms can effectively inhibit bacterial growth by various aspects. Usually, the main active mechanism involved is the ability of 2D-Ms to damage the bacterial cell membrane physically. The ultra-fine edges of thin nanolayers of CB-2D-Ms allow them to easily penetrate the bacterial cell membrane, causing structural damage and leakage of intracellular components. This disruption of the bacterial membrane ultimately leads to cell death. Another advantage of CB-2D-Ms is their high

surface area-to-volume ratio, which allows them to interact with bacterial cells effectively. This property is particularly useful in the case of biofilms, which are notoriously difficult to eradicate using conventional antibiotics. By physically disrupting the biofilm structure and inducing oxidative stress, CB-2D-Ms can effectively inhibit biofilm formation and growth. In addition to their physical action, CB-2D-Ms also exhibit antimicrobial activity through chemical interactions with bacterial cells. For instance, Nanda *et al.* mentioned that GO can disrupt bacterial membrane potential and induce oxidative stress, leading to bacterial death.<sup>82</sup> Similarly, Wang *et al.* observed that under solar irradiation,  $\text{C}_3\text{N}_4$  inhibited bacterial growth by generating ROS, which can damage bacterial DNA and proteins.<sup>83</sup> Fig. 6 shows a schematic representation of the mechanism of action of CB-2D-Ms. Furthermore, using CB-2D-Ms as antibacterial agents offers several advantages over traditional antibiotics. These materials demonstrate a wide spectrum of action against several bacteria, as well as drug-resistant bacteria. Additionally, CB-2D-Ms exhibit low cytotoxicity towards mammalian cells, making them excellent candidates for medical applications.

In general, we can say that the mode of action of CB-2D-Ms for antibacterial applications involves both physical and chemical interactions with bacterial cells. Their unique properties, such as high surface area-to-volume ratio and broad-spectrum activity, effectively inhibit bacterial growth and biofilm formation. With further research, 2D materials have the potential to become an important class of antibacterial agents for a wide range of applications.

Table 1 Different CB-2D-Ms and their antibacterial applications.

### 4. Ways to improve biocidal activity of CB-2D-Ms

CB-2D-Ms have remarkable physico-chemical characteristics, which makes them next-generation materials for numerous applications including biocidal agents. We observed that CB-2D-Ms themselves have less biocidal activity but due to their tunability and easy surface functionalization, they are extensively used for biomedical applications including biocidal agents.<sup>31,85–87</sup> In this aspect, researchers have developed a newer strategy to improve biocidal activity by incorporating metals/metal oxides, polymers, and surface functional groups to produce CB-2D-Ms-based hybrid materials. Here we discuss some important aspects of improved biocidal activity. (1) Size: smaller-sized (length) CB-2D-Ms have better biocidal activity compared with larger sizes, as smaller-sized CB-2D-Ms easily penetrate bacterial cell walls. (2) Surface texture: the surface texture of CB-2D-Ms is one of their important characteristics, as rough and porous texture significantly improves the adhesion of bacteria, thereby improving biocidal activity. (3) Surface engineering: incorporating functional groups on the surface of CB-2D-Ms (modulating their surface charge) considerably enhances their affinity towards bacterial membranes, thereby increasing interaction that leads to cellular disruption and subsequently cell death. (4) Modification: incorporation of

Table 1 Different CB-2D-Ms and their biocidal activities

No.	CB-2D-M	Method of synthesis	Bacterial strain	Ref.
1.	Graphene	Hydrothermal	<i>E. coli</i> , <i>S. typhimurium</i> , <i>Enterococcus faecalis</i> , and <i>B. subtilis</i>	49
2.	Ag/graphene composite	Chemical reduction	<i>E. coli</i>	48
3.	ZnO decorated graphene	Hydrothermal	<i>E. coli</i> and <i>S. typhi</i>	46
4.	Graphene	Green synthesis	<i>E. coli</i> and <i>P. aeruginosa</i>	45
5.	Gallic acid-GO	Modified Hummers' method followed by stirring	<i>S. aureus</i>	52
6.	GO-CuO	Modified Hummers' method followed by reduction and immobilization	<i>P. syringae</i> pv. <i>tomato</i>	53
7.	GO-guanidine	Modified Hummers' method followed by covalent conjugation	<i>E. coli</i> and <i>S. aureus</i>	54
8.	GO-Ag	Modified Hummers' method followed by ultrasonication	<i>E. coli</i> , <i>S. aureus</i> , <i>S. epidermidis</i> , and <i>C. albicans</i>	55
9.	GO-Ag	Modified Hummers' method followed by a reduction	<i>E. coli</i> and <i>S. aureus</i>	56
10.	Agphosphomolybdate/rGO	Modified Hummers' method followed by hydrothermal method	<i>E. coli</i>	84
11.	Ag-rGO	Microwave irradiation (MWI) and UV light irradiation	<i>E. coli</i>	59
12.	Phosphorus/sulfur-C <sub>3</sub> N <sub>4</sub>	Two-step template-free method	<i>E. coli</i> and <i>S. aureus</i>	64
13.	Fe <sup>3+</sup> -doped alkalinized C <sub>3</sub> N <sub>4</sub>	Calcination	<i>P. aeruginosa</i> , <i>E. coli</i> , and <i>S. aureus</i>	70
14.	GO-g-C <sub>3</sub> N <sub>4</sub>	Reduction and heating	<i>E. Coli</i>	65
15.	CeO <sub>2</sub> -g-C <sub>3</sub> N <sub>4</sub>	Hydrothermal	<i>S. abonyi</i> , <i>E. Coli</i> , <i>S. aureus</i> and <i>B. Cereus</i>	66
16.	g-C <sub>3</sub> N <sub>4</sub> -Cr-ZnO	Co-precipitation	<i>E. Coli</i> , <i>Bacillus subtilis</i> , <i>S. aureus</i> , and <i>S. salivarius</i>	71
17.	g-C <sub>3</sub> N <sub>4</sub> -membrane	Acid etching	<i>E. coli</i>	69
18.	MXene	Chemical etching	MRSA	77
19.	Colloidal MXene	Chemical etching followed by sonication	<i>E. coli</i> and <i>Bacillus subtilis</i>	17

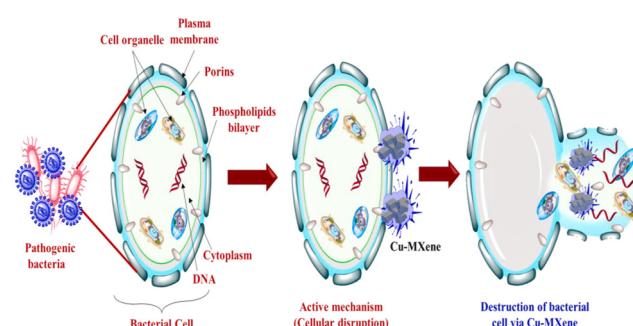


Fig. 6 Schematic representation of the mechanism of action of CB-2D-Ms Reproduced from ref. 81 with permission from Elsevier, copyright 2023.

metals/metal oxides and polymers within CB-2D-Ms *via* in-situ and ex-situ doping. Usually, metals/metal oxides like Cu, Zn, Ag, Au, *etc.*, and polymers like chitosan, polyethylene glycol, *etc.*, have antibacterial ability.<sup>88–92</sup> Although metals/metal oxides have strong antibacterial activity, higher concentrations lead to cellular toxicity. Incorporating metals/metal oxides within CB-2D-Ms significantly improved their biocidal activity with insignificant toxicity. It is important to mention that the biocompatibility of the materials is an important factor and we need to design effective biocidal agents by using these strategies with high biocompatibility.

## 5. Conclusion and future prospects

In conclusion, CB-2D-Ms are competitive antimicrobial agents. However, challenges are related to the stability and large-scale application of CB-2D-Ms. Moreover, the stability and the performance efficacy depend on the thickness, size, and

functionality of the surface of CB-2D-Ms. Additionally, different synthesis approaches have been adopted to control the various factors subsequent to their performance.

Further, functional CB-2D-Ms with various encapsulated agents to increase the interlayer spacing or adhere on the surface of CB-2D-Ms might provide novel ideas to address the stability challenges and foster antimicrobial activity. Moreover, incorporating metals/metal oxides, polymers, and surface functional groups might improve the antibacterial efficiency of CB-2D-Ms. Studies might provide general guidelines for tuning physico-chemical properties that are required for the concept of antibiotic materials. Additionally, in-depth mechanism of action is required to understand the molecular as well as cellular aspects between micro-organisms and CB-2D-Ms. In summary, this review deepens the knowledge of CB-2D-Ms and their properties to facilitate biocidal activity, which can help to reveal the hidden properties of available 2D-Ms and novel CB-2D-Ms for various biomedical applications including antibiotic materials as well as in wound healing. However, challenges still need to be addressed in the development of CB-2D-Ms for antibacterial applications. These include improving the stability and biocompatibility of the materials, optimizing their antibacterial properties, and ensuring their long-term safety. In conclusion, CB-2D-Ms have shown great potential for antibacterial applications due to their unique properties and intrinsic antibacterial activity. Further research is needed to fully realize the potential of CB-2D-Ms for antibacterial applications and address the challenges associated with their development.

## Author contributions

NT & MA conceptualized design, wrote, and revised the manuscript. DC wrote the original draft.



## Conflicts of interest

There are no conflicts to declare.

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