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Bactericidal capacity of Nano-composite against Multi-drug resistant bacteria associated with nosocomial infections in adult ICUs

S.A.Abdelkader[1], H.S.Elsheshtawy[2], M.M. Amer[3], S.S.Abd El-Salam[3] and D.D.Ibrahim[4]
[1]Microbiology Dept., at Faculty of dentistry, Sinai Univ., Sinai, Egypt,
[2]Microbiology and Biotechnology Dept., Egyptian petroleum researching institute (EPRI),
[3]Microbiology, Botany Dept., Faculty of Science, Benha Univ., Benha, Egypt,
[4]Researcher, Catalysis depat., Egyptian petroleum researching institute (EPRI).
E-mail: sanaa.atef18@fsc.bu.edu.eg

Abstract

Nosocomial infections are those that occur in a healthcare facility or hospital environment and are known as hospital-acquired infections (HAIs). Pseudomonas aeruginosa, E. coli, Klebsiella pneumonia, Enterococci spp, Methicillin-resistant Staphylococcus aureus (MRSA), and many other bacteria are the primary causes of HAI. Resistance in bacteria can be overcome only by the intelligent and practical deployment of nanotechnology. Antibacterial resistance has cleared the path for more effective and sensitive ways for detecting and treating bacterial infections because to Nanotechnology. These nano-composites have been utilised with molecular beacons to determine bactericidal actions, target medication delivery, and anti-fouling coatings, among other purposes. More recent approaches to improving efficacy against MDR bacteria, such as combining more than one nanoparticle with polymer (Nano-composites), have also been summarised. Nano-composite may be used to fight multidrug-resistant bacteria in a novel way, according to our findings.

Keywords: Hospital-acquired infection, Multidrug resistance bacteria, Nanotechnology, Nosocomial infection, Nano- composite.

1. Introduction

Seriously sick patients needing advanced airway, respiratory, cardiac, and renal support are admitted to the intensive care unit (ICU). Because of several factors, such as nosocomial infections, which are the most common problems among hospitalised patients and have the greatest fatality rates among those in intensive care units, mortality and morbidity remain high. Exogenous and endogenous sources are both possible for bacteria in the natural flora. When the immune system of the host is compromised, an opportunistic bacterial infection may emerge. Bacteria that belong to the Gram-positive phylum include coagulase-negative Staphylococci, S. aureus, Streptococcus spp., and Enterobacteriaceae (e.g. faecalis, faecium). C.difficile is the most usually reported pathogen in US hospitals related with HAI (15 percent of all infections with a reported pathogen). [1][5] Common Gram-negative organisms include Klebsiella pneumoniae and Klebsiella oxytoca, Escherichia coli, Proteus mirabilis, and Enterobacter species; Acinetobacter baumanii, Burkholderinia cepacian, and Pseudomonas aeruginosa. Because of its intrinsic multi-drug resistance, Acinetobacter baumanii is linked to significant mortality in the critical care environment. [7][8] Multidrug-resistant bacteria are frequent in HAI and are linked with high mortality. [9] Approximately 20% of all reported pathogens had multidrug-resistant patterns, according to one research. [10] In addition to MRSA and VRSA, there are also methicillin-resistant Staphylococcus aureus (VISA), vancomycin-resistant Staphylococcus aureus (VRSA), Enterobacteriaceae with extended-spectrum cephalosporin resistance consistent with ESBL production, vancomycin-resistant Enterobacteriaceae (VRE), carbapenem-resistant Enterobacteriaceae, and multidrug resistant Pseudomonas aerugin (MDR Pseudomonas spp.).

In poor and developing countries, the spread of antibiotic resistance is exacerbated by the fact that antibiotics may be purchased over the counter without a prescription, while access to medicines in developed countries was considerably more restricted [32].

Antibiotic overuse by healthcare personnel was formerly assumed to be the primary cause of the emergence of multi-drug resistant organisms and microbial resistance.

Antibacterial agents are substances that either kill or limit the development of bacteria (bactericidal agents) (bacteriostatic agent). If you're looking for an ideal antibacterial agent, it should be effective against a wide variety of G+ and G germs, have a long lasting antibacterial effect, low resistance, be safe and not influence the physical and chemical characteristics of the carrier, and have minimal adverse effects. [11]

Inorganic antibacterial materials have gained a lot of attention in recent years for a variety of applications12. Metal ion-based inorganic materials, such as ceramics, zirconium and calcium phosphates, and glasses, make up the majority of the antibacterial inorganic materials. Fiber, ceramic, plastic, composites, construction materials, and surface coatings are all examples of applications for inorganic antibacterial material. In recent years, antibacterial glasses have been a major focus of study. In general, glass is a material that can withstand a wide range of chemicals because of its network structure. However, its chemical durability may be reduced by modifying the chemical makeup of the substance. Dissolving slowly or rapidly in water, phosphate-based glasses (PBGs) are biodegradable because of their chemical makeup. For example, binary sodium phosphate glasses may dissolve fully within minutes of contact with purified water. [13] In addition, the deterioration rate of these glasses may be customised to fit the final use. 14 Because of their high solubility in metal ions and amorphous nature, phosphate glass melts have an advantage over other oxide glasses (such as silicate glass) when it comes to handling large concentrations of metal ions. There is no distinct phase for the metals since they are part of the phosphate glass structure. Consequently, when

the glass degrades, the release of ions is regulated, and their rate of release is determined by the total degradation rate of the material. Antibacterial metal ions, such as Ag+, Cu2+, or Zn+, may be delivered using PBGs, which have a greater reactivity than silicate glasses (silica-based glasses are very resistant to degradation). Glasses that melt at very low temperatures may result in partial or total fusion with polymers with high melting points, which can lead to exceptionally homogenous distribution in the polymer when combined with such high melting point polymer. When manufacturing polymer-glass composite materials, such as biocidal fibres, a fusion of the glasses may be created. Antibacterial glasses may be made by combining the ability to make glass with limited chemical durability with the trait that glass can retain metal ions. These antibacterial metal ions may be added to various glass systems and have been demonstrated to have an impact on bacteria and fungi's development. 15-18 To offer an antibacterial effect when in contact with an aqueous medium or moisture, the glass slowly dissolves while also releasing the ions that give it its silver, copper, or zinc colour. Most antibacterial glasses may either be made by adding an antibacterial chemical to the glass batch before to fabrication or by post-treatment techniques, such as ion exchange or surface coating. Because of their broad variety of uses, such as cosmetics, electrical appliances, textiles, and biomaterials, as well as wastewater treatment, antibacterial glasses are becoming more and more significant in recent years. Glass particles, like zeolite, may be readily incorporated into fibres and coatings using traditional processes. Antibacterial glasses that don't need any particular precautions or preparation conditions were the primary goal of this research. They can be managed in terms of dissolving rates. Ag2o-60P2O5–20CaO–20Na2O and Ag2o-60P2O5–30CaO–10Na2O were chosen as the basis sodium calcium phosphate glasses for this application.

2. Materials And Methods

1. Samples Collection:

Between April 2019 and December 2020, researchers in Egypt's intensive care units (ICUs) gathered seventy different bacterial isolates. Patients, tools, air, floor, wall, and medical personnel were all sampled. The specimens were swiftly transferred to the Microbiology Laboratory at Benha University's Faculty of Science in accordance with (25) [20] in aseptic circumstances.

2.Isolation, Identification and Characterization of bacterial isolates:

Bacterial isolates were isolated and streaked for several consecutive times on nutrient agar medium until pure single colonies were obtained. Preliminary identification of bacteria was based on colonial morphology of the organisms such as hemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from primary cultures for identification and characterization of the isolates.

The purified cultures of the selected multi-drug resistant isolates were identified and confirmed after investigating morphological cultural characters and biochemical tests according to standard clinical laboratory methods reported and recommended by Bergey's Manual of determinative bacteriology [21]and others (25)[22].

3. Antibiotic susceptibility tests:

Thirteen of different antibiotics were selected based on the pharmacological action and what antibiotic targets in bacterial cells and each one of them representing different group for carrying out the antimicrobial susceptibility test. The antibiotic discs used in this research were purchased from Oxoid Ltd., England. The name of antibiotic discs, the code, the potency and the standard evaluation of inhibition zones were tested according to [28][23]

Antibiotic susceptibility test for the bacterial isolates was carried out by Kirby Bauer disc diffusion technique according to [29][24]. Mueller-Hinton agar was used for testing the sensitivity of the experimental isolates to the different antibiotics[30][25] **.**The appropriate antibiotics were placed in the agar aseptically using sterile forceps the plates were then incubated at 37°C for 24hours.

4. Identification of bacterial isolates:

4.1. The purified cultures of the selected multi-drug resistant isolates were identified and confirmed after investigating morphological cultural characters and biochemical tests according to standard clinical laboratory methods reported and recommended by Bergey's Manual of determinative bacteriology [31][26] and others .

4.2. Identification by VITEK® MS (MALDI-TOF) technology.

Is an automated mass spectrometry microbial identification system that uses Matrix Assisted Laser Desorption Ionization Time-of-Flight .

How Maldi-Tof works:-

A. The target slide is prepared and introduced to a high-vacuum environment.
B. A precise laser burst ionizes the sample.
C. A "cloud" of proteins is released and accelerated by an electric charge.
D. After passing through the ring electrode, the proteins' Time of Flight is recorded using a formula from the time recorded.
E. Proteins are detected with a sensor to create a spectrum that represents the protein makeup of each sample.

5. Studing the antimicrobial activity of the different synthesized Nano-composites on the multidrug resistant bacteria:

5.1 Antibacterial activity test

The antibacterial activity of un doped and silver-doped P_2O_5–CaO–Na_2O glasses was evaluated against bacterial species(*S. aureus ,E. coli ,Klebsiella pneumonia ,Enterococcus fecalis and P. aeruginosa* using the agar-disk diffusion assays. The antibacterial activity was deduced from the inhibition zone diameter (IZD), zone of no bacterial growth, measured under the stated experimental conditions. The antibacterial activity increases with the increase in IZD and vice versa.

5. 1. Preparation of different Nano-composites solutions:

10 mg of powder nano-composite was dissolved with 1ml of

distilled boiled water, Also repeated with 1ml of Dimethyl-sulfate (DMS) and 1ml of Dimethyl -formamide (DMF) respectively in a sterile tube with continuous shaking and mix through vortex.

2. Assay of the antibacterial activity of different Nano-composites solutions against the selected multi-drug resistant strains:

1. Muller-Hinton agar medium was sterilized by autoclaving at 121ºC for 15 minutes, cooled to 45 ºC and inoculated with the multi resistant isolates by striking the swab over the surface of the medium on three directions to confirm a complete distribution. The density of the bacterial suspension equivalent to that of standard barium sulphate (0.5 McFarland).
2. 1ml of suspension (100M) of different nano-composite solutions was transferred to the wells which made in agar surface. The used DMS, DMFand distilled water wells served as negative controls.
3. The plates were kept in refrigerator for two hours for diffusion of the antibacterial substances.
4. The plates were then incubated for 24 hours at 37ºC**.**
5. After incubation the entire diameter of the inhibition zone was measured.

3. Results and Discussion

Nosocomial infections are frequent complications of hospitalizations. In this study seventy isolates of bacteria were collected from patients at hospital's intensive care units (ICUs). The selected isolates include 40 (57.1%) isolates from males and 30 (42.9%) isolates from females whose ages ranged between 25 to 75 years were reported a significant relationship between age and nosocomial infections.

1.Antibiotic suscebtibility test :

1.1 Frequency of different bacterial groups within multi-drug resistant bacterial isolates:

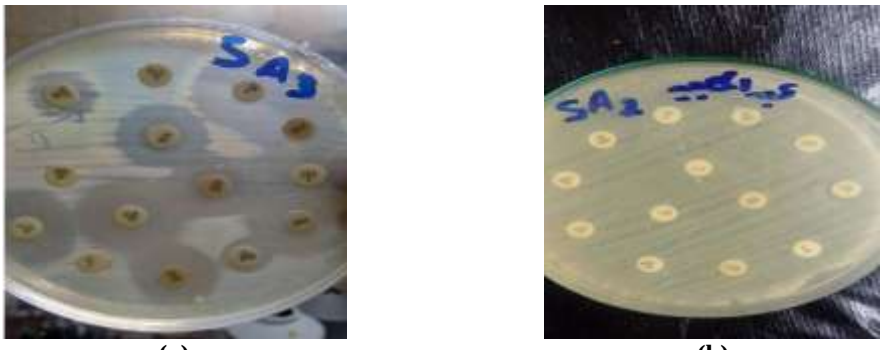
In our study(table 2) ,the multi-drug resistant (MDR) bacterial isolates (38) isolates were distributed as 23 gram negative bacterial isolates (60.5%) and 15 gram positive bacterial isolates (39.5%), and divided into five groups namely, *Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus fecalis*. *Escherichia coli* was the most frequent pathogen within MDR isolates representing 31.6% of MDR isolates followed by *Staphylococcus aureus* with frequency percentage 26.3% followed by *Pseudomonas aeruginosa* and *Klebsiellapneumoniae* with frequency percentage 21.05,13.2 % respectively. On the other hand *Enterococcus fecalis* was the less frequent pathogen within MDR isolates (7.9%). [27] found that the microorganisms most commonly isolated from clinical specimens were *E. coli* (28%), *S. aureus*(11.11%) and *P .aeruginosa* (8.6%). [28] reported that the most causative agents was *E. coli* (87%of cases) followed by *K. pneumonia* (10%). But in other study nosocomial infections were most frequently caused by *Acinetobacter* (34.5%), followed by *Pseudomonas* (32.8%), *Klebsiella* (13.9%), *E. coli* (12.1%) and *Citrobacter* (5%) [29]

Escherichia coli infection is one of the major public health problems in many developing countries and has contributed exceedingly to morbidity, mortality and increased health costs .[30] *K.pneumoniae* pneumonae causes a severe, rapid-onset illness that often causes areas of destruction in the lung and causes less serious respiratory infections, such as bronchitis, which is usually a hospital-acquired infection [31]. Staphylococcus *aureus* is well documented as a human opportunistic pathogen and one of the most frequently identified pathogens in clinical laboratories[32].*S. aureus* is notorious for its ability to develop broad antibiotic resistance. The most commonly known resistance of *S. aureus* is (MRSA), the new strain resistant to vancomycin, (VRSA), this organism poses a major threat to human health globally**[**33]

Pseudomonas aeruginosa is identified as one of the most common pathogen causing hospital acquired infections **[**34]

Table (1) Frequency of different bacterial groups within multi-drug resistant bacterial isolates

Group no.	Bacterial species	Isolates no.	Total no.	Percentage (%)
I	***Escherichia coli***	5,9,17,21,23,27,28,37,40,53,55 and60	12	31.6
II	***Staphylococcus aureus***	2,10,16,20 ,25,38,45, 49,50 and 61	10	26.3
III	***Pseudomonas aeruginosa***	13,15 ,27,28,33,42,56 and 63	8	21.05
IV	***Klebsiella pneumonia***	22,24,46.48 and 70	5	13.2
V	***Enterococcus fecalis***	19,25 and 65	3	7.9
Total			38	100



(a) **(b)**

Photo (1.1) antibiotic susceptibility test (a), sensitive isolate and (b), resistant isolate.

2. Identification of multi-drug resistant bacterial isolates

The multi-drug resistant bacterial isolates (38isolates) which are resistant to 95% or more of selected antibiotics were selected and then identified.

Different morphological, physiological and biochemical tests were conducted to identify the multi-drug resistant bacterial isolates to the genus and species levels. The obtained results are tabulated in table (2) . The staining reactions and the culture characteristics of the isolates on simple, enriched and selective media were recorded. According to the keys of identification protocols the tested isolates were divided into five groups as following:

Group I: ***Escherichia coli*** , Group II: ***Klebsiella pneumonia*** , Group III: ***Pseudomonas aeruginosa***,

Group IV: ***Staphylococcus aureus*** ,and Group IV:***Enterococcus fecalis***.

Table (2) Morphological characteristics, biochemical tests and confirmatory tests for identification of 38 multi-drug resistant bacterial isolates

Test	Group I	Group II	Group III	Group IV	Group V
Morphological characters:					
Gram's stain	**- ve**	**- ve**	**- ve**	**+ ve**	**+ ve**
Shape	**Bacilli**	**Bacilli**	**Bacilli**	**Cocci**	**Cocci**
Arrangement	**Short rods**	**Short rods**	**Rods**	**Irregular Clusters**	**Chains**
Colonies characters	smooth, convex, moist, translucent, gray with a shiny surface ,entire edge and Pink colonies on MacConkey	Elevated and mucoid appearance Pink colonies on MacConkey agar	large, smooth, with flat edges, elevated center and produce blue green pigment on nutrient agar	Raised, smooth, glistening, translucent, with entire margins. Pigmentation varies from gray to yellow to orange.	It produces smooth, gray, non-hemolytic translucent colonies (rarely produces α or β hemolysis) on Blood agar.
Motility	**+ ve**	**- ve**	**+ ve**	**- ve**	**-ve**
Physiological characters:					
Oxidase	**- ve**	**- ve**	**+ ve**	**- ve**	**-ve**
Catalase	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**-ve**
Coagulase	**- ve**	**- ve**	**- ve**	**+ ve**	**-ve**
Indole	**+ ve**	**- ve**	**- ve**	**- ve**	**-ve**
MR	**+ ve**	**- ve**	**+ ve**	**+ ve**	**-ve**
VP	**- ve**	**+ ve**	**- ve**	**+ ve**	**+ ve**
Citrate	**- ve**	**+ ve**	**+ ve**	**- ve**	**- ve**
H2S production	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**
Urease	**- ve**	**+ ve**	**+ ve**	**+ ve**	**-ve**
DNase at 25^{O}C	**- ve**	**- ve**	**- ve**	**+ ve**	**-ve**
Nitrate reduction	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ve**
Gelatin Liquefaction	**- ve**	**- ve**	**+ ve**	**- ve**	**+ve**
Tellurite reduction	**- ve**	**- ve**	**- ve**	**+ ve**	**-ve**
Blood hemolysis	**γ-hemolysis**	**γ-hemolysis**	**β-hemolysis**	**β-hemolysis**	**γ-hemolysis**
Pyocyanin and Pyoverdin production	**- ve**	**- ve**	**+ ve**	**- ve**	**+ve**
Fermentation of:					
D-Glucose	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**
Lactose	**+ ve**	**+ ve**	**- ve**	**+ ve**	**+ ve**
Sucrose	**+ ve**	**+ ve**	**- ve**	**+ ve**	**+ve**
D- sorbitol	**+ ve**	**+ ve**	**- ve**	**- ve**	**+ve**
Maltose	**+ ve**	**+ ve**	**- ve**	**+ ve**	**+ve**
D-Mannitol	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ve**
D-Mannose	**+ ve**	**+ ve**	**- ve**	**+ ve**	**+ve**
D-Xylose	**+ ve**	**+ ve**	**- ve**	**- ve**	**+ve**
Identification	***E. coli***	***K. pneumonia***	***P. aeruginosa***	***S. aureus***	***E.fecales***

3. The antimicrobial activity of the different synthesized Nano- composites on the multidrug resistant bacteria:

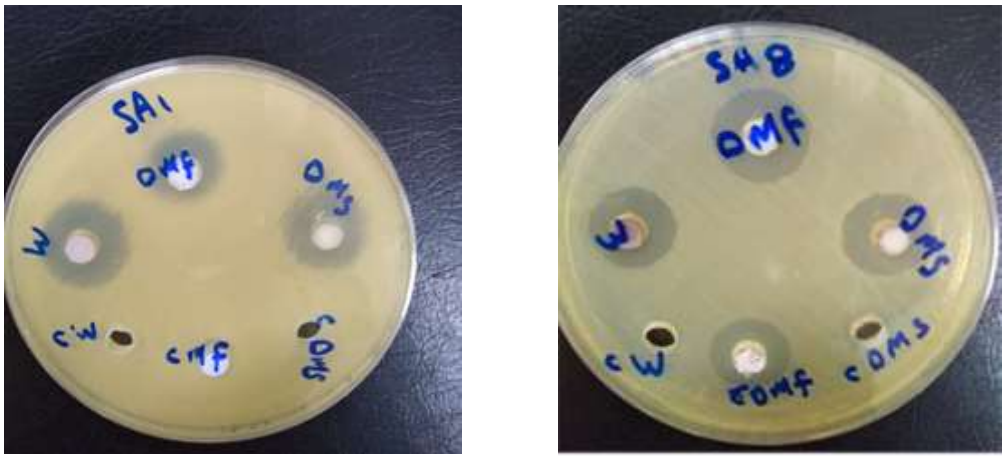
Table (3.1) Antimicrobial activity of the different synthesized Nano-composites on multi-drug resistant bacterial isolates.

	Water Control (µg/ml)	Sample (µg/ml)	DMF Control (µg/ml)	Sample (µg/ml)	DMS Control (µg/ml)	Sample (µg/ml)
Klebsiella pneumonia 59	**0**	**17**	**0**	**15**	**0**	**16**
Enterococcus fecalis 35	**0**	**10**	**0**	**0**	**0**	**10**
Staphilococcus.aureus 18	**0**	**16**	**0**	**17**	**0**	**15**
Escherichia coli 55	**0**	**15**	**0**	**15**	**0**	**14**
Pseudomonas aeruginosa 42	**0**	**16**	**0**	**14**	**0**	**12**

For the most resistant bacterial isolates, the disc diffusion method was used to identify the most resistant bacterial isolates, such as E. coli (55), K pneumoniae (59), P aeruginosa (42),S. aureus (18) and E. fecalis (35).

Different Nano-composites solutions were tested on the most resistant bacterial strains to see what impact they had. Table (3.1) and figure (2.1) indicated that all bacterial isolates were resistant to the solvent control and that it had no impact on the bacterial isolates, demonstrating this clearly (water,DMF,DMS). Nano-composite solutions (water, DMF, DMS) had an impact on bacterial isolates on the other hand. For K. pneumoniae (59), S. aureus (18), P. aeruginosa (42), E. coli (55) and E. fecalis (35) the sensitivity of water Nano-composite solution was found to be 17,16,16,15and 10 g/ml. A nano-composite solution of DMF was found to be 17, 15, 15, 14 and 0, respectively, for the bacteria S. aureus (18), E. coli (55) and K pneumoniae (59) as well as for the bacteria E.fecalis (35). (table 3.1and figure 2.1). Finally, the concentrations of DMS nano-composite solution for K. pneumoniae (59), S. aureus (18), E. coli (55),P. aeruginosa (42) and E.fecalis (35) were17, 15, 15, 14 and 0 g/ml, respectively, for K. pneumoniae, S. aureus, E. coli, P. aeruginosa and E.fecalis (table 3.1 and figure 2.1). More or less, this is in line with what I expected (34)

If NPs are absorbed by bacteria, they may release metallic and non-metal ions into the surrounding media and/or attach to the negatively charged functional groups on the membrane of the bacterial cell. Adsorption of silver ions (from silver NPs) on the cell membrane, for example, results in protein coagulation [36]. This synergy between several toxic pathways may be responsible for the antibacterial action of graphene oxide/Cu/Ag NPs on E. coli, P. aeruginosa, K. pneumoniae, S. aureus, and Methicillin-resistant S. aureus (MRSA) [37].



Antimicrobial activity of the different synthesized Nano- Photo(2.1) composites on multi-drug resistant bacterial isolates.

4. Conclusion

It is clear that certain of the hospital isolates in Egypt might constitute a major health concern, as it has been shown and proved. Poor hand hygiene, cross-contamination between patients and medical staff, and the abuse of drugs may all contribute to their frequency and prevalence.

Results from this work showed that 60P2O5–20CaO–20Na2O nanocomposites might be used as bactericidal agents against both non-MDR and MDR bacteria. Disc diffusion, tolerance determination, and time-kill were used to measure the antibacterial activity of these nanocomposites. The findings show that nanocomposites are more effective at killing bacteria. Ag+ has an active part in bactericidal effects by generating ROS and demonstrating its position as an active species. Nanocomposite Ag2O - 60P2O5-20CaO–20Na2O and Ag2O-60P2O5–30CaO–10Na2O nanocomposites implanted in synthetic polymers must be studied further before being used in the medical and healthcare sectors, however.

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