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Experimental Research

Prevalence of multidrug-resistant strains in device associated nosocomial infection and their in vitro killing by nanocomposites

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ABSTRACT

Background: As per WHO, global burden of healthcare-associated infections (HAIs) ranges between 7% and 12%. There is a dire need to screen Device associated nosocomial infections (DANIs) in hospitals(1). To investigate the prevalence of microbes in hospitals in DANI cases and analyse in vitro control of multi-drug resistant strains by nanotechnology intervention.

Methods: Patients diagnosed with DANI were enrolled and monitored. Identification and antibiotic susceptibility pattern of the etiological agent of DANIs were made by the phenotypic method and Vitek 2 automated systems according to standard protocol. In addition, biosynthesized nanocomposite was analysed for their antimicrobial activity by agar well-diffusion method, CFU count and DNA degradation analysis.

Results: There were a total of 324 patients diagnosed with DANIs. Total 369 microbial pathogens were isolated from DANI patients. The majority (87%) of the pathogenic microbes were gram-negative bacilli and all were multidrug-resistant. 41.5% of the gram-negative isolates were ESBL producers. Methicillin-resistant *Staphylococcus aureus* contributes about 7.3% of the total isolates in gram-positive bacteria. Nanocomposite showed 100% bactericidal activity at 5 mg/ml concentration within 3 h of incubation, whereas 2.5 mg/ml concentration of nanocomposites takes 6 h to inhibit complete growth.

Conclusions: DANI, which was found in patients of all age groups, us due to multidrug-resistant gram-negative bacteria. The most commn causative agents were *Acinetobacter baumannii* and *Citrobacter* species. Nanocomposites can provide an alternative solution to prevent the DANIs.

1. Introduction

Patients requiring life-saving devices are perpetually admitted to the hospital's Intensive Care Unit (ICU). They regularly go through invasive strategies together with intra-tracheal intubation for mechanical ventilation or insertion of intravascular and urinary catheters. In these instances, if the right care package isn't followed, there will be development of device-associated Nosocomial Infections (DANIs). Increased nosocomial infections leads to excessive morbidity and mortality. Incidences of infections amongst patients inside the ICU are 5- to 7-fold higher than trendy inpatient admissions of all nosocomial infections in a hospital [1]. There is a worldwide escalation in each community- and hospital obtained infections because of Antimicrobial-resistant (AMR) microorganisms compromising the capacity to deal with those sufferers effectively, thereby underscoring the need for endured surveillance, suitable prescribing of antibiotics, implementation and implementation adherence to stringent contamination control measures [2].

Several reviews describe the epidemiology and microbiology of ICU-obtained nosocomial infections, which include Ventilator-associated Pneumonia (VAP), Central Line-associated Bloodstream Infection (CLABSI), and Catheter-associated Urinary Tract Infection (CAUTI) [3].

Abbreviations: Antimicrobial-resistant, AMR; Intensive Care Unit, ICU; Device-associated Nosocomial Infections, DANIs; Ventilator-associated Pneumonia, VAP; Central Line-associated Bloodstream Infection, CLABSI; Catheter-associated Urinary Tract Infection, CAUTI.

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Studies have proven the occurrence of pathogens, along with the resistant genotypes of Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococcus, Extended-Spectrum Beta-Lactamase (ESBL)-generating *Escherichia coli* and *Klebsiella* species& carbapenems-resistant *E.coli*, *Klebsiella* speciesProteus species, *Pseudomonas aeruginosa*, *and Acinetobacter baumannii* inflict HAIs, especially inside the ICU setting. Available healing alternatives for AMR organisms are seriously restrained as those organisms often showcase multidrugresistance. It is well known that inappropriate and irrational use of antibiotics to treat infections leads to the emergence of Multi Drug Resistant (MDR) strains among the common bacterial isolates [4]. This translates into a prolonged hospital stay, a significant increase in morbidity and mortality, and an escalating economic burden. The frequency of infections among patients admitted to the ICU may vary from one geographical region to another, from one hospital to another, and even among the ICUs within one hospital. The type of infection, the profile of pathogens causing these infections, their antimicrobial susceptibility patterns also vary according to the location. It is, therefore, imperative for the treating clinician to have adequate information of the spectrum of microorganisms and the AMR patterns prevalent in that particular setting for initiating empirical therapy with appropriate antimicrobial agents [5].

Nanomaterials inhibit bacterial growth or activity that results in infections. Nanoparticles penetrate the bacteria and biofilm leading to reactive oxygen species (ROS) generation that eliminates bacteria. Thus, nanoparticles are a novel approach to combat drug-resistant bacterial infections [6]. Additionally, the ionic activity of nanoparticles can modulate the bacterial signal transduction leading to the inhibition of bacterial growth or inactivating the enzymes by interacting with them [7]. In the present study, an attempt was made to analyse antibacterial effect of nanocomposite against MDR strains and its potential use in combat against nosocomial infections. This study was conducted in MMIMSR and associated hospital, a tertiary care teaching hospital located in the Mullana, Ambala Haryana, India, with an active infection control committee. We studied the prevalence of VAP, CLABSI and CAUTI, causative organism, antimicrobial resistance, and MRSA prevalence in *S.aureus*, ESBL and Metallo-β-Lactamase (MBL) producing Gram-Negative Bacteria (GNB). Additionally, we have also analysed antibacterial susceptibility of carbon quantum dots decorated dual Z scheme Manganese Indium Sulphide/Cuprous Oxide/Silver oxide against MDR strains.

2. Material & methods

Prospective, site-specific surveillance of DANI was carried out from October 2018 to July 2021 in various ICUs of Hospitals in Maharishi Markandeshwar (Deemed to be University), Mullana,-Ambala, India. The approval was taken from Institutional Human Research Ethics Committee (IEC no. 1147). Based on the CDC's National Nosocomial Infections Surveillance (NNIS) system criteria, samples for three DANIs: ventilator-associated pneumonia (VAP), catheter-associated urinary tract infection (CA-UTI) and central line-associated bloodstream infection (CLABSI) were taken into consideration.

2.1. Selection criteria for patients

2.1.1. Patients selection

For VAP the Patient with pneumonia and placed on Mechanical Ventilation for >2 calendar days was selected as per CDC guidelines, including onset of purulent sputum or change in the character of sputum, increased respiratory secretions, increased suctioning requirements, new onset or worsening cough, dyspnea, tachypnea, bronchial breath sounds, worsening gas exchange, increased oxygen requirements, or increased ventilator demand [8]. Similarly CDC guidelines were followed for enrolling patients for CAUTI and CLABSI [10,11]. In all the cases only those patients were considered for DANI where the symptoms appeared after two calendar days of admission in ICU [9,10].

2.1.2. Sample collection and identification of microbes

From patients suspected of DANI, the appropriate clinical samples were collected and microbial analysis was performed with the help of a trained Infection Control Nurse the samples were collected as per clinical and laboratory standards institute (CLSI) guidelines. All the clinically relevant details were collected from patients, including age and sex. For microbial analysis of VAP, deep tracheal aspirate from the endotracheal tube was collected. For CAUTI, urine samples were aseptically aspirated from the sampling port of the urinary catheter. For CLABSI, the peripheral blood sample, the central line removed aseptically, and the distal 5 cm of the catheter were collected and processed for analysis(11).

According to the laboratory Standard Operational Procedures, phenotypic and automated identification of microbes was made based on CLSI recommendations. Phenotypic identification consisted of Gram staining followed by a series of biochemical tests specific for each group of microorganisms. Yeasts were isolated on Sabouraud agar and identified by culturing on CHROM agar based on their colonies' colour, texture, and shape. Moreover, we have employed an automated VITEK® 2 system to identify strains. Samples exhibiting microbial growth on blood agar or MacConkey agar were inoculated into specific identification cards of the automated VITEK® 2 system [12].

2.1.3. Antibiotic susceptibility test (AST) of bacterial isolates

AST of the identified strains was done by Kirby Bauer disc diffusion method and VITEK® 2 automated systems using the standard protocol. Detection of MRSA strains was done using cefoxitin (30 μg) disc by modified Kirby-Bauer disc diffusion method [13]. The phenotypic analysis of extended-spectrum β Lactamase was done by Double-Disc Diffusion as described earlier [14]. Similarly, the phenotypic confirmation of Metallo β Lactamase was done by Combined Disc Method as described earlier [15].

2.1.4. Analysis of antimicrobial property of nanocomposite

We further wanted to test the effect of nanocomposites on the growth of multidrug-resistant strains. In our recent study, we found that nanocomposites could control the growth of bacterial strains. We tested the antibacterial activity of carbon quantum dots decorated dual Z-scheme Manganese Indium Sulphide/Cuprous Oxide/Silver oxide Nanocomposites using the agar well diffusion assay and by enumerating the CFUs as described earlier. Different concentration of nanocomposite (1.25 mg/ml, 2.5 mg/ml & 5 mg/ml) was used [16–18].

2.1.5. Effect of nanocomposites on genomic DNA of MDR bacterial strains

MDR strains (0.1 OD_{600}) were incubated with and without nanocomposite (2.5 mg/ml) at 37 °C overnight [19]. After the incubation, cells were harvested for genomic DNA isolation as described earlier [20]. Isolated DNA was analysed and compared with the untreated sample by agarose gel electrophoresis.

3. Results

3.1. Prevalence of DANI

During our study from October 2018 to July 2021, 7050 patients were admitted in different ICUs with indwelling devices. There were 2160 patients with an endotracheal tube carrying a mechanical ventilator, 1590 patients with a central venous catheter, and 3300 patients with a urinary catheter. The total number of device days of 7050 patients was 64800. Based on clinical signs and symptoms in correlation with microbial culture (discussed below), 324 patients were diagnosed with DANI (124 patients of VAP, 18 patients of CLABSI, and 180 patients with CAUTI). During the study period, CAUTI was the most commonly

diagnosed DANI found in 180 patients with 3.75 cases per 1000 device days, followed by VAP in 126 patients with 11.21 cases per 1000 device days and CLABSI at 18 patients with 3.18 cases per 1000 device days. The crude infections rate of DANIs was 5 cases per 1000 device days (Table I). The age distribution as shown in Table II indicated that DANI was more prevalent in 18–40 age groups.

3.2. Identification of microbes causing DANI

The samples were processed as described in Materials and Methods to know the responsible microbial pathogens of DANI by standard tests. We found 369 microbial pathogens from 324 DANI patients. *Acinetobacter baumannii* was the most common microbial pathogen contributing 23.57% of the total isolates followed by *Citrobacter* species 17.88%, *Klebsiella* species 57%, and *Escherichia coli* 13%. Other microbial pathogens isolated from DANI patients were *Staphylococcus aureus* 10.84% (68.28% MRSA & 35.72% MSSA), *Enterococcus* species 1.62% and *Proteus* species 0.81%. Candida was the only fungal isolate contributing in DANI. *Proteus* was the most minor contributor in DANI (Table III).

3.3. Antimicrobial sensitivity pattern of gram-positive cocci isolated from DANI patients

Further, we wanted to analyse the drug sensitivity pattern of the isolated pathogens. Our results indicated that all gram-positive isolates were sensitive to linezolid and vancomycin. MRSA strains showed sensitivity to G and TE at 55% and 44%, respectively. The MRSA showed the least sensitivity to E, AZ & OF with 0%, 0%, and 11%. MSSA strains showed sensitivity to all antibiotics tested. *Enterococcus* species showed 50% sensitivity to COT as well (Table IV).

3.4. Antimicrobial sensitivity pattern of gram-negative bacilli isolated from DANI patients

This study found that tigecyclinewas the most effective antibiotic against all gram-negative bacterial isolates. PIT, IMP, MRP GEN and AK also showed notable sensitivity, in contrast to this cephalosporin group of antibiotics (CTR, CTX, CAZ& CPM) showed the least sensitivity to all gram-negative bacilli. Specifically, in the case of *Escherichia coli* IMP, MRP, GEN, AK, AMC and PIT showed a sensitivity of 93.75%, 87.50%, 81.5%, 68.75%, 68.75% and 81%, respectively. In case of *Klebsiella species*IMP, PIT, AMC, MRP, GEN and AMC showed sensitivity of 73.68%, 73.68%, 63.1% 57.89%, 57.89% and 63.15% respectively. In case of *Proteus*species only TGC, IMP and MRP showed 100% sensitivity rest antibiotics were resistant. In case of *Pseudomonas aeruginosa* IMP, MRP, GEN, AK, PIT, and CTR showed sensitivity of 92.85%, 92.85%, 78.57%, 78.57%, 78.57% and 71.42% respectively. In case of *Acinetobacter baumannii* IMP, MRP, AK and GEN showed sensitivity of 82.75%, 75.86%, 58.62% and 55.17% respectively. In case of *Citrobacter*species IMP, PIT and AMC showed sensitivity of 86.36%, 59.09% and 54.54% respectively (Table V). Besides MSSA, all the bacterial isolates were MDR.

Table 1
Prevalence of Device associated infections (VAP, CLABSI & CAUTI).

DANIs Parameter	Total no. of DAI patients	Total no. of device days	DAIs rate/1000 Device days
VAP	126	11238	11.21
CLABSI	18	5652	3.18
CAUTI	180	47910	3.75
Total	324	64800	5

Table 2
Distribution of Age, sex of ICUs patients with DANIs.

DANI	Age			Gender (M/F)
	18–40 years	40–60 years	>60 years	
VAP	78	33	15	66/60
CLABSI	9	6	3	9/9
CAUTI	87	57	36	129/51
Total	174	96	54	204/120

Table 3
Distribution of microbial pathogens isolated from DANI patients.

		VAP	CLABSI	CAUTI	Total
Staphylococcus aureus	*MRSA*	12	0	15	27
	MSSA	6	0	9	15
Enterococcus species		0	0	6	6
Escherichia coli		6	0	42	48
Klebsiella species		33	0	24	57
Proteus		0	3	0	3
Pseudomonas aeruginosa		18	3	21	42
Acinetobacter baumannii		48	6	33	87
Citrobacter species		39	3	24	66
Candida species		0	3	15	18
Total		162	18	189	369

Table 4
Antimicrobial Sensitivity pattern gram-positive bacteria isolated from DANI patients.

Antibiotics	Name of Isolates		
	Staphylococcusaureus		Enterococcus
	MRSA	MSSA	
E	0%	0%	N/A
AZM	0%	0%	N/A
GEN	55%	100%	N/A
AK	22%	100%	N/A
TE	44%	80%	N/A
LZ	100%	100%	100%
LE	33%	60%	0%
CIP	33%	60%	0%
OF	11%	100%	0%
NX	33%	80%	0%
COT	55%	60%	50%
V	100%	100%	100%
HLG* (120 μl)	N/A	N/A	50%
HLS* (300 μl)	N/A	N/A	50%

E: erythromycin; AZM: azithromycin: GEN: gentamycin; AK: amikacin; TE: tetracycline; LZ: linezolid; LE: levofloxacin; OF: ofloxacin; NX: norfloxacin; COT: cotrimoxazole; V: vancomycin; HLG: high-level gentamycin; HLS: high-level streptomycin; N/A: not applied.

* **HLG** and **HLS** were only tested for *Enterococcus species* to detect synergistic response with beta-lactam drugs, showing 50% sensitivity.

3.5. Distribution of methicillin resistance staphylococcus obtained from DANI patients

Methicillin resistant S. *aureus* was detected in 64.28% of the total *S. aureus* whileremaining 35.72% of the staphylococcus was identified as methicillin sensitive *S. aureus*(Fig. 1 A).

3.6. Distribution of ESBL and MBL producer among gram-negative bacilli

Among total Bacterial isolates all strains of *Proteus*species (100%) and 68.75% of *Escherichia coli* were ESBL producer followed by *Klebsiella* species (57.89%), *Citrobacter*species (31.81%), *Acinetobacter baumannii* (27.58%) and *Pseudomonas aeruginosa* (21.42%). Metallo β- Lactamase was identified in 31.81% of *Citrobacter* species amongst total bacterial isolates followed by *Acinetobacter baumannii* (27.58%), *Klebsiella* species

Table 5
Antimicrobial Sensitivity Pattern of Gram-Negative bacteria obtained from DANI patients.

Antibiotics	*Escherichia coli*	*Klebsiella* species	*Proteus* species	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Citrobacter* species
MZ	0%	0%	0%	42.85%	0%	0%
AMC	68.75%	63.15%	0%	50%	31%	54.54%
PIT	81%	73.68%	0%	78.57%	13.79%	59.09%
CTR	6.25%	10.52%	0%	71.42%	3.44%	6.89%
CTX	6.25%	10.52%	0%	14.28%	13.79%	4.54%
CAZ	6.25%	10.52%	0%	42.85%	17.24%	4.54%
CPM	56.25%	21.05%	0%	21.42%	17.24%	10.34%
IMP	93.75%	73.68%	100%	92.85%	82.75%	86.36%
MRP	87.50%	57.89%	100%	92.85%	75.86%	48.27%
GEN	81.50%	57.89%	0%	78.57%	55.17%	36.36%
AK	68.75%	52.63%	0%	78.57%	58.62%	45.45%
TOB	25%	36.84%	0%	57.14%	34.48%	18.18%
TE	6.25%	31.57%	0%	64.28%	24.13%	27.27%
MI	6.25%	10.52%	0%	42.85%	17.24%	13.63%
CIP	18.75%	42.10%	0%	42.85%	20.68%	31.81%
OF	31.25%	15.78%	0%	50%	13.79%	22.72%
NX	6.25%	31.57%	0%	42.85%	34.48%	31.81%
COT	25%	15.78%	0%	28.57%	44.82%	18.18%
TGC	100%	100%	100%	100%	100%	100%

MZ: mezocillin; AMC: amoxicillin + clavulanic acid; PIT: piperacillin and tazobactum; CTR: ceftriaxone; CTX: cefotaxime; CAZ: ceftazidime; CPM: cefepime; IMP: imipenem; MRP: meropenem; GEN: gentamycin; AK: amikacin; TOB: tobramycin; TE: tetracycline; MI: minocycline; CIP: ciprofloxacin; OF: ofloxacin; NX: norfloxacin; COT: cotrimoxazole; TGC: tigecycline.

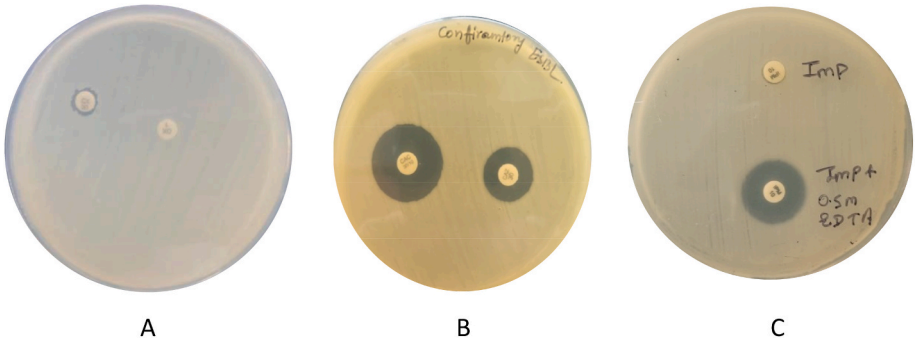

Fig. 1. (A) MRSA detection by cefoxitin disc diffusion method, (B) Double disc methods for ESBL detection, (C) Metallo β-Lactamase detection by combined disc method.

(26.31%) and *Pseudomonas aeruginosa* (7.14%). MBL was not detected in *Escherichia coli* and *Proteus*species (Figs. 1 and 2-B, C).

3.7. Antimicrobial activity of nanocomposites

The isolated strains from DANI patients were resistant to conventional drugs so, we further wanted to explore if nanocomposites could control these pathogens. Antimicrobial activity of nanocomposite was investigated against the resistant strains using the agar well diffusion assay and by enumerating CFUs in the presence of nanocomposites. Zones of inhibition (mm) around each well containing different concentration (0.25 mg/ml, 0.5 mg/ml, 1 mg/ml and 2 mg/ml) of nanocomposite and Colistin (10 μg) are represented in (Table VI). All MDR

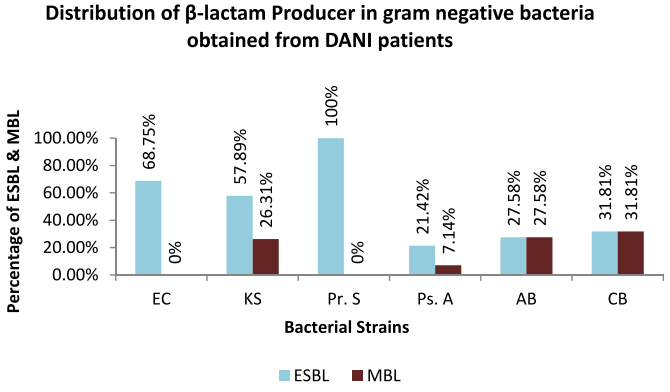

Fig. 2. Distribution of β-lactamase Producer. EC: *Escherichia coli;* KS: Klebsiella species; Pr. S: *Proteus species;* Ps. A: *Pseudomonas aeruginosa;* AB: *Acinetobacter baumannii;* CB: *Citrobacter species;* ESBL: Extended spectrum of β-lactamase; MBL: Metallo β-lactamase.

Table 6
Antimicrobial Activity of carbon quantum dots decorated dual Z-scheme Manganese Indium Sulphide/Cuprous Oxide/Silver oxide Nanocomposites against various MDR isolates after 24 h of incubation by agar well diffusion method.

MDR isolates	Zone of Inhibition at various concentrations				Colistin
	0.25 mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	10 μl/ml
Escherichia coli	17 mm	18 mm	21 mm	24 mm	17 mm
Klebsiella species	13 mm	17 mm	18 mm	20 mm	16 mm
Proteus species	15 mm	16 mm	18 mm	20 mm	Intrinsic resistant
Pseudomonas aeruginosa	13 mm	17 mm	18 mm	20 mm	16 mm
Acinetobacter species	17 mm	18 mm	20 mm	22 mm	17 mm

isolates tested by agar well diffusion assay showed zones of inhibition ranging 20 mm–24 mm (Table VI). Furthermore, we found complete bactericidal activity at 5 mg/ml of nanocomposite within 3 h of incubation. At a lower concentration of 2.5 mg/ml, complete growth inhibition was observed after 6 h whereas at the lowest concentration tested of 1.25 mg/ml the antibacterial activity was 85.5% in 6 h (Fig. 3).

3.8. DNA degradation by nanocomposite

One of the methods by which the nanocomposite could show bactericidal activity is by producing reactive oxygen species which could degrade DNA of MDR strains. To analyse this, bacterial strains were treated with 2.5 mg/ml nanocomposite at 37 °C for 6 h. After incubation DNA extraction was done and it was observed that bacterial strains treated with nanocomposite exhibited complete degradation of DNA (Fig. 4, lanes 2–6) as compared to the untreated bacterial strain (lane 1).

4. Discussion

During the study period, 7050 patients were hospitalized in the different ICUs for 64800 days. We found that 324 patients acquired DANI. The overall DANI rate was 5 per 1000 device days, whereas the overall rate of getting VAP, CLABSI & CAUTI in patients was 11.21, 3.18 and 3.75 per 1000 device days, respectively. Similarly, Deorukhkar et al., showed DANI rate of 2.1 per 1000 device days [21], Khan D.I. et al., 2016 observed DANI rate 4.7 per 1000 device days [22]. Lower DANIs rate had been achieved by applying a proper bundle care approach and hygiene. In contrast to our study, Kumar et al., 2017 observed DANI rate 18.3 per 1000 device days [23], Bammigatti et al., 2017 showed overall DANI rates of 74.9 per 1000 device days, it was due to longer duration of ICU stay serves greater exposure to pathogens, frequent invasive procedures, lack of a proper hospital infection control and monitoring system. Moreover, with respect to gender male patients developed DANIs higher than female patients but there seems no relation between age and gender with respect to development of DANIs [24].

A total of 369 pathogens were isolated from DANI patients. Majority (82.11%) were gram-negative organisms, the rest, 11.38%, were gram-positive and 6.51% were yeast (*Candida* sp.) were the etiological agent. All of the isolated microbial pathogens showed resistance to at least one antibiotic of three to four groups of antibiotics and considered as multidrug-resistant bacterial strains. According to previous study infections due to gram-positive organism are more prominent in the Western world ICUs, in contrast to this, gram-negative organism were the major contributor in causing DANI in India and Asia-Pacific region [25].

The National Nosocomial Infections Surveillance System reported a significant increase in the proportion of *Acinetobacter* species in causing hospital acquired infections among all gram-negative aerobes [26]. *Acinetobacter baumannii* was also the predominant (23.57%) bacterial pathogen isolated form DANI patients in our study. Interestingly, we found that except MSSA, all the bacterial isolates exhibited MDR phenotype. In brief *Acinetobacter baumannii* and *Citrobacter* species showed 100% sensitivity to TGC followed by IMP, MRP, GEN, PIT and AK. Similar to our findings, Ghanshani R et al., 2016 revealed in their study that ≥95% bacterial pathogens were sensitive to colistin, *Klebsiella* and *Pseudomonas* were >50% resistant to 3rd generation cephalosporin and carbapenems, while *E. coli* was still >50% sensitive to carbapenems and *Acinetobacter*> 50% sensitive to 3rd generation cephalosporin. Gram-positive organisms showed zero sensitivity to penicillin, oxacillin, and tetracycline. MSSA were 100% sensitive to vancomycin, and 50% sensitive to linezolid and gentamycin. Enterococcus was 100% sensitive to linezolid, 50% sensitive to vancomycin [27]. Study conducted by Dutta V et al., 2017 found that *S. aureus* and *Enterococcus* were 100% sensitive to linezolid and vancomycin and more than 50% resistant to gentamicin, erythromycin, and ciprofloxacin. Indiscriminate use of antibiotics for prolonged and inappropriate duration is the possible explanation of such high levels of multidrug resistance in these organisms [28].

In addition to this, 68.75% *Escherichia coli*, 57.89% *Klebsiella* species, 100% *Proteus* species, 21.42% *Pseudomonas aeruginosa,* 27.58% *Acinetobacter baumannii and* 31.81% *Citrobacter* species were ESBL producers. This suggested that cephalosporin group of antibiotics were the most favored drug of first-line treatment and use of these antibiotics leads evolution of ESBL production by microbial pathogens resulting in their lower efficacy. Also, 31.81% *Citrobacter* species, 27.58% *A. baumannii,* 26.31% *Klebsiella* species and 7.14% *Pseudomonas aeruginosa* was MBL producer. We found that *Escherichia coli &Proteus* sp. didn't produce MBL. Mathai et al., showed 3.6% strains of Enterobacteriaceae were MBL producer [29]while Patro S et al., 2018 observed thatMBL was positive in 17.64% non-fermenters and 17.39% in *Enterobacteriaceae* [30]. Due to their efficacy of broad spectra and low toxicity β-lactam antibiotics are the major bulk of prescribed antibiotics in ICUs across the globe. However, irrational use of beta lactam antibiotics had been resulted in the development and spread of drug resistant bacterial pathogens mainly by production of ESBL enzymes. For the treatment of ESBL producing pathogens carbapenems group of antibiotics were the drug of choice hence frequent use of carbapenems, pathogens rapidly adapt and modify it self to produce Carbapenemase which becomes major healthcare burden [31].

Nowadays, research in the area of nanotechnology indicates the use of nanocomposite in controlling bacterial infections. These nano composites can be used in drug deliver as well as in making nanomaterials to control infections [7]. We found that carbon quantum dots decorated dual Z-scheme Manganese Indium Sulphide/Cuprous Oxide/Silver oxide nanocomposite was able to control the MDR strains. It efficiently kills the bacteria within 3 h at 5 mg/ml concentration by degrading its DNA. Nanoparticles/nanocomposite penetrate the bacteria leading to reactive oxygen species generation that eliminates bacteria. Coating nanocomposite on surface of medical devices such as endotracheal tube, central line catheter, and urinary catheter may impede microbes responsible for the development of DANIs and provide a great tool to combat drug-resistant bacterial infections. Different nanomaterials, such as nanoparticles and nanotubes can be directly used in biomedical devices to prevent spreading infections.

5. Conclusions

An intensive care setting is a high-risk area for acquiring DANIs. The high rate of infections in ICUs is due to frequent use of medical devices, increased device days, length of hospital stays, and patient disease severity. Our study suggested for an alarming increase in MDR in DANI patients which indicate that there is a dire need to control these MDR.

Ethical approval

All procedure for research has been approved by the ethics committee of IEC (institutional ethical clearance) number: 1147.

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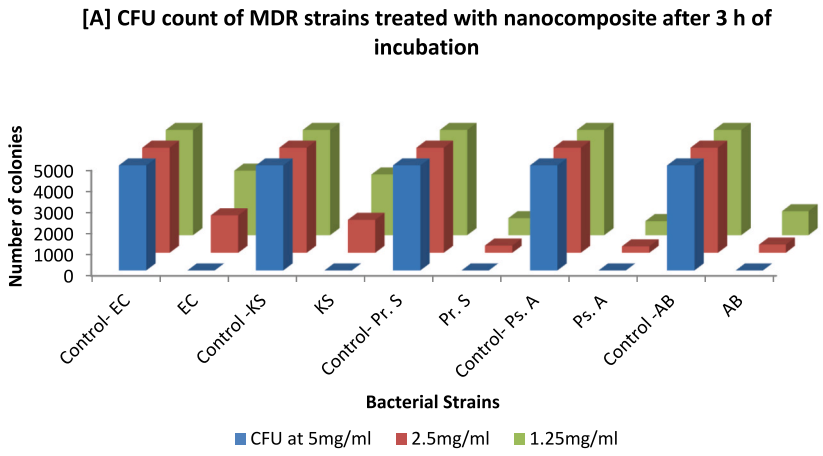
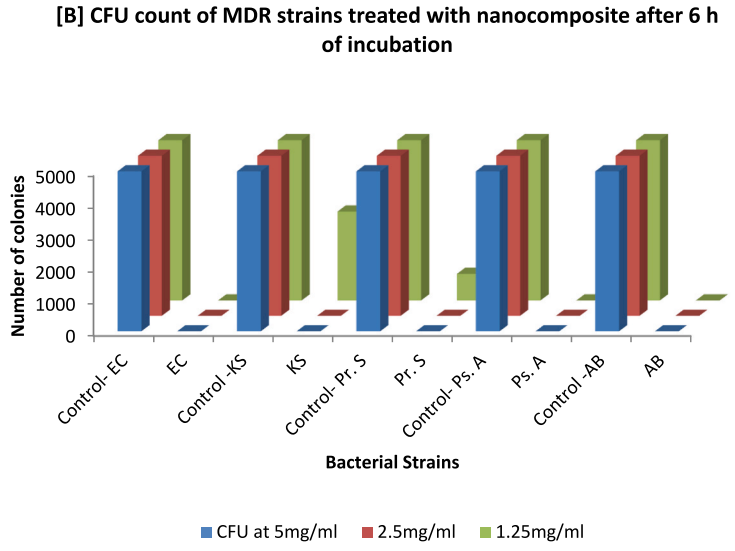
Consent

None.

Author contribution

Shahbaz Aman: conceptualisation and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript,





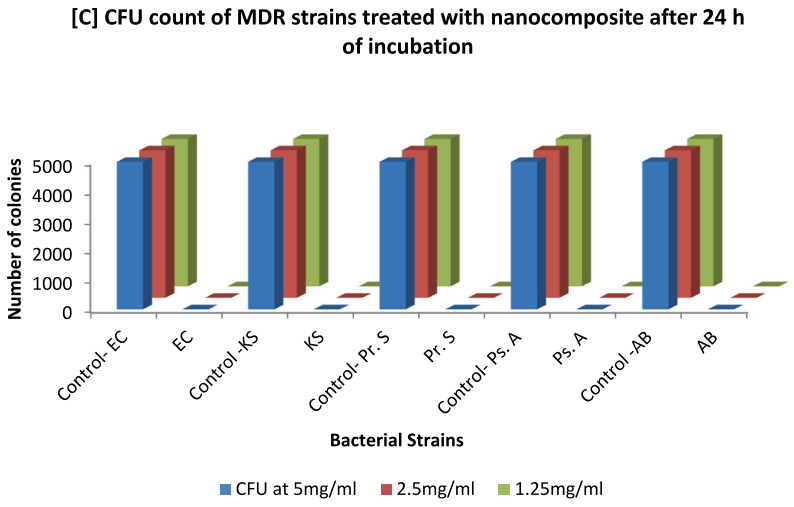


Fig. 3. Antimicrobial Activity of nanocomposites. Carbon quantum dots decorated dual Z-scheme Manganese Indium Sulphide/Cuprous Oxide/Silver oxide Nanocomposite was incubated with various MDR isolates and CFUs was enumerated at different time intervals (A) 3 h (B) 6 h (C) 24 h. EC: *Escherichia coli*; KS: *Klebsiella species*; Pr. S: *Proteus species*; Ps. A: *Pseudomonas species*; AB: *Acinetobacter baumannii.*

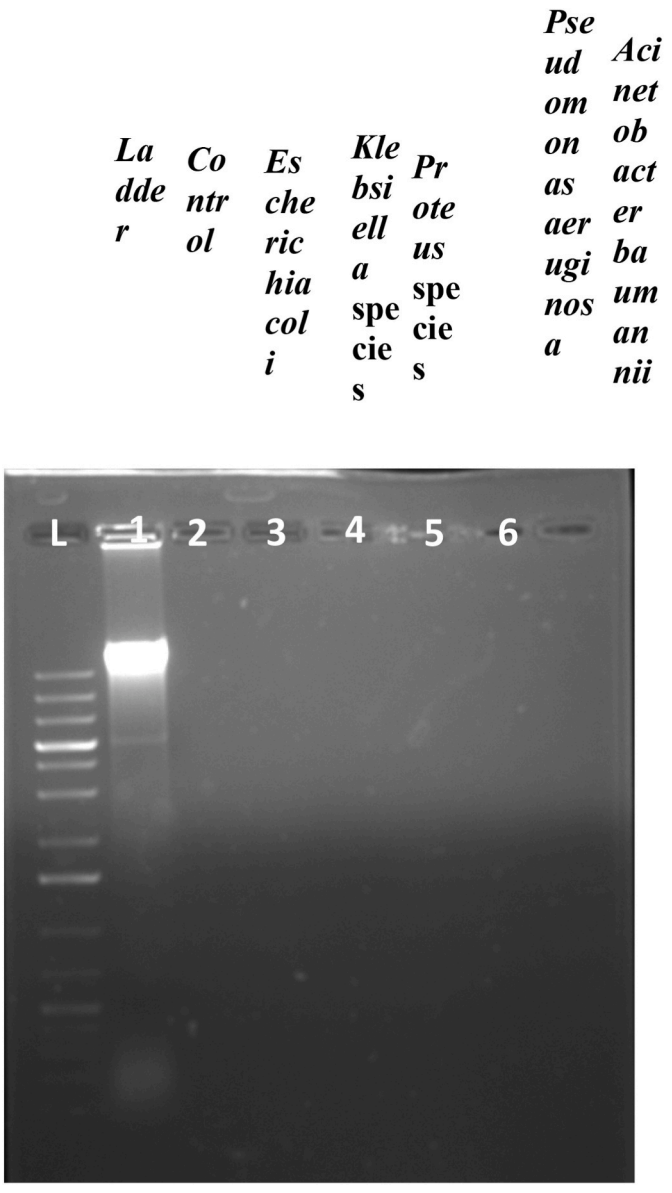


Fig. 4. Agarose gel electrophoresis for DNA degradation by 2.5 mg/ml nanocomposite: Lane L (2 kb Ladder), Lane 1 (DNA isolated from untreated MDR *Escherichia coli strains*), Lane 2–6 (DNA isolated from treated MDR strains of *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*).

Narinder Kaur: Coordinated and supervised data collection, Divya Mittal: carried out the initial analyses, and reviewed and revised the manuscript, Shalini Shriwastav: data collection, data interpretation, Shubham Chauhan: data collection, data interpretation, Pardeep Singh: data collection, data interpretation, Sheetal Sharma: resources, data collection, data interpretation, Reena V. Saini: reviewed and revised the manuscript, Hardeep Singh Tuli: data interpretation and writing manuscript, Adesh K. Saini: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Registration of research studies

Name of the registry:

Unique Identifying number or registration ID:

Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Professor Adesh K. Saini.
Professor Narinder Kaur.

Declaration of competing interest

None.

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