DOI: 10.7860/JCDR/2022/58018.17065

Original Article

Microbiology Section

Efficacy of Green Synthesised Iron Oxide Nanoparticles against Various Uropathogens: A Cross-sectional Study

RAHUL HARIKUMAR LATHAKUMARI[1], SUJITH RAVI[2], SHUBHANGI TRISAL[3], LEELA KAKITHAKARA VAJRAVELU[4], DALIA VISHNUDASAN[5], JAYAPRAKASH THULUKANAM[6], BALAMURALI VENKATESAN[7]

ABSTRACT

Introduction: The shoot up of antimicrobial resistance leading to the Multidrug Resistance (MDR) phenomenon in clinical pathogens has forced us to develop novel technologies to cease this global threat immediately. Iron oxide nanoparticles can be a breakthrough solution to this dilemma due to its magnetic properties and biocompatibility. Non toxic and biocompatible applications of magnetic nanoparticles can be enriched further by special surface coating with organic or inorganic molecules.

Aim: To determine the antibacterial activity of green synthesised iron oxide nanoparticles against various clinical isolates.

Materials and Methods: This was a cross-sectional study conducted from June 2021 to April 2022. This study was conducted at the Department of Microbiology, SRM Medical College Hospital and Research Centre (SRMMCH&RC), Kattankulathur, Chengalpattu, Tamil Nadu, India. Nanoparticles underwent surface modifications and characterisation using X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX), Ultraviolet (UV) Visible Absorption Spectra, and Fourier-Transform Infrared Spectroscopy (FTIR) followed by charge characterisation through agarose gel electrophoresis. Kirby-Bauer Disc Diffusion method was used for screening the sensitivity and resistance pattern of 50 selected isolates and Minimum Inhibitory Concentration (MIC) was assessed using MIC Microbroth Dilution technique with the help of resazurin. Tukey post-hoc multiple comparisons test to analyse the zone of inhibition of antibacterial efficacy.

Results: Out of the four different concentrations of bare and coated nanoparticles (0.0375 mg/mL, 0.07 mg/mL, 0.15 mg/mL, 0.3 mg/mL), bare nanoparticles inhibited the growth of Methicillin Resistant *Staphylococcus aureus* (MRSA) at 0.3 mg/mL while citrate coated nanoparticles inhibited the growth at 0.15 mg/mL, 0.018 mg/mL, 0.0375 mg/mL, 0.07 mg/mL, and 0.15 mg/mL dilutions were used in case of Carbapenem-resistant *Klebsiella pneumoniae* (CR *K. pneumoniae*) and MDR *Escherichia coli*, from which both organisms were inhibited at 0.15 mg/mL of bare and coated nanoparticles.

Conclusion: Iron nanoparticles synthesised from the marine algae *Chaetomorpha antennina* could be used in the future as a drug carrier or as an antimicrobial agent.

Keywords: Antibacterial activity, Iron oxide nanoparticles, Minimum inhibitory concentration, Trisodium citrate

INTRODUCTION

The inception and expansion of pathogens with new mechanisms of drug resistance are continuing to intimidate our ability to diagnose and treat common infections. Extensive use of antibiotics and misuse of them are the prime reason for antibiotic resistance in bacteria [1]. Among which MRSA, MDR *E. coli*, CR *K. pneumoniae* are the significant global health issue that causes morbidity and mortality worldwide. In recent years, MRSA has led to many nosocomial infections responsible for deadly conditions like necrotising fasciitis, osteomyelitis, sepsis, toxic shock syndrome and endocarditis. Penicillin-binding protein (PBP2a) encoded by *mecA* or *mecC* is accountable for MRSA. Vancomycin has been used as the choice of drug for decades but certain isolates with complete or intermediate resistance have emerged recently [2].

One of the most frequent pathogens encountered in clinical settings is *E. coli*, which is associated with nosocomial and community acquired infections, now it has developed resistance to the broad spectrum of antimicrobial agents. Many strains of *E. coli* are producers of β-lactamases such as Extended Spectrum β-Lactamases (ESBL), AmpC β-lactamases and Metallo β-Lactamases (MBL). The Centre for Disease Control and prevention (CDC) defines MDR as the ability of an organism to be resistant to atleast one agent in three or more antimicrobial groups [3]. Among these, the most common one is MDR *E. coli* [4].

Currently, *Klebsiella pneumoniae* is an important cause of healthcare-associated infections. Many strains carry plasmids that encode MDR due to the synthesis of carbapenemases and MBL. Due to the production of these enzymes, antibiotics coming under the carbapenem group cannot work efficiently. In this condition, the polymixin and colistin is used as the last line of therapy. Moreover, colistin resistant strains are increasing day by day [5].

An important area of modern science is nanotechnology, which is concerned with the study of and manipulation of matter at a scale between 1 and 100 nanometers [6]. Synthesis of nanoparticles using biosystems such as plants, algae, bacteria, yeast, fungi and actinomycetes were termed as green synthesis. The perks of green nanotechnology is an alternative, well organised, economical, and eco-friendly method for the synthesis of nanoparticles with specified properties [7]. Because of their magnetic properties and biocompatibility, iron oxide nanoparticles have been widely used as bacterial separation agents, contrast agents for bio imaging, and magnetic hyperthermia agents. They can lose their surface properties when exposed to the environment and can result in particle aggregation, change in charge and other surface properties. Therefore, surface modification using sodium citrate will be useful to avoid such problems [8]. This study tries to utilise the ability of nanoparticles to disrupt the bacterial cell by the production of Reactive Oxygen Species (ROS) [9]. Since, urinary tract infections are the most common nosocomial infections and the selected organisms were commonly associated with such infections, this study focuses on the evaluation of antibacterial activity of iron oxide nanoparticles against uropathogens such as MRSA, CR *K. pneumoniae* and MDR *E. coli.*

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Microbiology, SRM Medical College Hospital and Research Centre (SRMMCH&RC), Kattankulathur, Chengalpattu, Tamil Nadu, India. A total of 50 resistant isolates were isolated from urine specimens collected from SRMMCH&RC during a total time period of June 2021 to April 2022. Out of which eight MRSA, 22 CR *K. pneumoniae* and 20 MDR *E. coli* were obtained. The study was approved by the Institutional Ethical Committee (IEC) and ethical clearance was obtained (IEC.No 2898/IEC/2021). Similar studies were insufficient in order to calculate sample size, so authors collected as much as isolates from hospital (50 resistant isolates from urine samples during the mentioned period). As only bacterial isolates were utilised, individual patients' consent were not required for the study.

Inclusion criteria: Fifty (50) MRSA, CR *K. pneumoniae*, and MDR *E. coli* isolates from urine samples collected before initiation of antibiotic therapy were included in this study.

Exclusion criteria: Isolates other than MRSA, CR *K. pneumoniae*, and MDR *E. coli* from clinical samples isolates collected after the initiation of antibiotic therapy were excluded in this study.

Synthesis of Nanoparticles

Synthesis of iron oxide nanoparticles from the marine green algae *Chaetomorpha antennina* was done by using previous study conducted at Amrita School of Biotechnology, Kollam Kerala, India, 0.1 g of algal powder along with ultrapure water was heated to 70-80°C. The crude algal extract was centrifuged and the supernatant thus obtained was used as the seaweed bioextract. 0.1 M $FeCl_2.4H_2O$ and 0.1% algal extract were added in the ratio of 2:3, in four different sets, each with a different pH. The pH of the reactions was adjusted to 6, 8, 10, and 12 using NaOH and the effect of pH on the nanoparticle properties were studied. These reactions were maintained at temperatures ranging from 60-70°C. The synthesised nanoparticle was washed thrice using 70% ethanol. It was kept in a hot air oven for 24 hours and dried. To evaluate the effect of temperature on the nanoparticle synthesis, two different sets of reactions were performed at a temperature ranging from 60-70°C and at room temperature. The green synthesised nanoparticle was washed and dried for storage [10].

Surface modification of iron oxide nanoparticles: The nanoparticle was coated with trisodium citrate ($Na_3C_6H_5O_7$) (0.01 M) by adding the trisodium citrate solution to the sonicated bare FeNp. Sonication were performed frequently to disperse nanoparticles into water [10].

Characterisation of Nanoparticles

Characterisation of the Fe_3O_4 nanoparticles was undertaken by the following methods.

UV-visible absorption spectra: Shimadzu's UV-2540s equipped with an ISR-240A sphere assembly were used to measure UV-visible absorption spectroscopy measurements in aqueous buffer in the range 300-800 nm. A quartz cuvette with a path length of 1 cm was used to scan the absorbance from 190-900 nm.

X-ray diffraction: With the help of X-ray powder diffraction, the phase composition of Fe_3O_4 nanoparticles was investigated over the range of 20-600 at a rate of five nanometers per minute (14, 1.54060 A). Using the Debye-Scherrer equation, one can predict the average particle size of Fe_3O_4 nanoparticles by relating its peak broadening in XRD to particle size. The following equation illustrates this relationship:

$$d=\lambda k/\cos\theta x\beta$$

Fourier Transform Infrared Spectroscopy (FTIR): The Fe_3O_4 -NP characteristics like surface bond were analysed using a FTIR spectrometer.

Scanning Electron Microscopy (SEM): A SEM study was carried out on nanoparticles synthesised at pH 8 and structural data was recorded and it enables the visualisation of nanoparticle's morphological characteristics.

Characterisation of FeNp based on charge: The charge of Fe_3O_4-nanoparticles was determined using Agarose Gel Electrophoresis (AGE). 1X Tris-acetate-EDTA (Ethylenediaminetetraacetic Acid) buffer was used to prepare a 2.5 percentage of agarose gel. A 100 V voltage was applied to the gel to run the samples [11].

Selection of isolates using Antibiotic Susceptibility Test (AST): Clinical isolates such as MRSA, MDR *E. coli* and CR *K. pneumoniae* are screened using the Kirby-Bauer technique on Mueller-Hinton agar [12]. The Clinical and Laboratory Standards Institute (CLSI) guidelines for 2021 were used to interpret the zone sizes [3].

Minimum Inhibitory Concentration (MIC) evaluation: The MIC of green synthesised iron oxide nanoparticles (Fe_3O_4) was evaluated using the broth microdilution technique with the help of 96 microtitre plates along with resazurin dye (0.015%). The 1/150 dilution of bacterial suspension were prepared from 0.5 McFarland standardised inoculum in Mueller-Hinton broth and 50 µL of this solution was transferred to each well except first column of microtitre plate (keep the first column of microtitre plate empty). A 3 mg/mL and 6 mg/mL nanoparticle:distilled H_2O solution was formulated for enterobacteriaceae and MRSA respectively [13].

STATISTICAL ANALYSIS

Tukey post-hoc multiple comparisons test to analyse the zone of inhibition of antibacterial efficacy.

RESULTS

A total of 50 isolate comprising 8 (16%) MRSA, 22 (44%) CR *K. pneumoniae*, and 20 (40%) MDR *E. coli* were isolated from the laboratory. Male to female ratio was found to be 6:2, 9:13, and 12:8 for MRSA, CR *K. pneumoniae* and MDR *E. coli* respectively. The maximum number of CR *K. pneumoniae* was isolated from the Medical Intensive Care Unit (MICU) ward 7 (31.8%) followed by Urology 4 (18.2%), General Medicine 4 (18.2%), Coronavirus Disease-2019 (COVID-19) Intensive Care Unit (ICU) 2 (9.1%), Electronic Intensive Care Unit (EICU) 2 (9.1%), Respiratory ICU 1 (4.5%), Labour ward 1 (4.5%) and casualty 1 (4.5%). Most of the MRSA was isolated from the MICU 4 (50%) followed by Surgical Intensive Care Unit (SICU) 3 (37.5%) and Respiratory Intensive Care Unit (RICU) 1 (12.5%). Majority of MDR *E. coli* were isolated from General Medicine 7 (35%) followed by Nephrology 3 (15%), Casualty 3 (15%), General Surgery 3 (15%), Urology 2 (10%), Neuroscience Intensive Care Unit (NSICU) 1 (5%) and MICU 1 (5%) [Table/Fig-1].

Antibiotic resistance pattern: The MRSA had the highest resistance to penicillin (100%), cefoxitin (100%) bacitracin (100%), and erythromycin (87%) and was susceptible to antibiotics such as novobiocin, clindamycin, tetracycline, linezolid, chloramphenicol, and vancomycin. CR *K. pneumoniae* showed resistance to most of the antibiotics and had 100% resistance to antibiotics of group penicillins, β lactams+β lactam inhibitors, 1st generation cephalosporins, carbapenems and aminoglycosides. The lowest resistance was observed in nitrofurans (68%), sulphonamides (68%) and cefuroxime (68%). All the MDR *E. coli* isolates were AmpC producers and carbapenem-resistant. They showed 100% resistance to β lactams, β lactam+inhibitors, all generation cephalosporins, and carbapenems except meropenem. The lowest resistance was observed in aminoglycosides and nitrofurans [Table/Fig-2-4].

Iron Nanoparticle Characterisation

UV-visible absorption spectra: The Surface Plasmon Resonance (SPR) of bare nanoparticles was found in between 190 and 250 nm which corresponds to that of magnetite. Near-infrared absorption indicates the complex to be Fe_3O_4, since Fe_2O_3 which also has two ionic species does not absorb in the near-infrared region. The presence of carbon nanoparticles could be appreciated using the

Characteristics	MRSA	CR *K. pneumoniae*	MDR *E. coli*
Number of isolates (n=50)	8 (16%)	22 (44%)	20 (40%)
Gender-wise distribution			
Male	6 (75%)	9 (40%)	12 (60%)
Female	2 (25%)	13 (60%)	8 (40%)
Age-wise distribution (years)			
≤18	-	-	-
19-60	3 (37.5%)	8 (36.3%)	13 (65%)
Above 60	5 (62.5%)	14 (63.6%)	7 (35%)
Ward-wise distribution			
MICU	4 (50%)	7 (31.8%)	1 (5%)
RICU	1 (12.5%)	1 (4.5%)	-
SICU	3 (37.5%)	-	-
LW	-	1 (4.5%)	-
UROW	-	4 (18.2%)	2 (10%)
GM	-	4 (18.2%)	7 (35%)
COVID ICU	-	2 (9.1%)	-
EICU	-	2 (9.1%)	-
Casualty	-	1 (4.5%)	3 (15)
Nephrology	-	-	3 (15%)
GS	-	-	3 (15%)
NSICU	-	-	1 (5%)

[Table/Fig-1]: Gender, age and ward-wise distribution of isolates.
MICU: Medical intensive care unit; RICU: Respiratory intensive care unit; SICU: Surgical intensive care unit; LW: Labour ward; UROW: Urology ward; GM: General medicine; EICU: Electronic intensive care unit; GS: General surgery; NSICU: Neuroscience intensive care unit

Antimicrobial agent	No. of resistant isolates	Resistance percentage (%)
Penicillin	8	100
Cefoxitin	8	100
Erythromycin	7	87
Clindamycin	1	12
Cotrimoxazole	1	12
Tetracycline	0	0
Linezolid	0	0
Ciprofloxacin	4	50
Chloramphenicol	0	0
Gentamicin	3	37
Teicoplaninn	0	0
Nitrofurantoin	0	0
Vancomycin	0	0
Bacitracin	8	100
Novobiocin	0	0

[Table/Fig-2]: Antibiogram of MRSA (N=8).

presence of a transient absorption band formed at wavelengths between 360 and 320 nm, 320 and 290 nm, 290 and 270 nm and 270 and 250 nm.

Fourier-transform infrared spectroscopy: Waves at 3,319 cm^{-1} correspond to OH stretching. The absorption band at 1,641 cm^{-1} indicates the presence of the C=O group around the nanoparticles. So, the synthesised nanoparticles were characterised as magnetite, since two absorption bands were observed at 307 cm^{-1} and 535 cm^{-1}.

X-ray diffraction: The angle between the transmitted and reflected beam was observed as 35.25° which is complementary to the crystallographic value of (311) of crystalline Fe_3O_4-nanoparticles. The results observed were highly acceptable and comparable with the standard value and sizes of magnetite nanoparticles. Hence, the crystallite size of the green synthesised iron oxide nanoparticles was identified as 6.625 nm using XRD along with the application of the Scherrer equation [Table/Fig-5].

Antimicrobial agent	No.of resistant isolates	Resistance percentage (%)
Ampicillin	22	100
Amoxicillin clavulanate	22	100
Ceftazidime	19	86
Ceftazidime with clavulanic acid	22	100
Cefepime	19	86
Cefuroxime	15	68
Ceftriaxone	19	86
Ertapenem	22	100
Meropenem	22	100
Piperacillin tazobactum	22	100
Amikacin	22	100
Ciprofloxacin	19	86
Cefotaxime	22	100
Cefoxitin	22	100
Cefazolin	22	100
Gentamicin	22	100
Imipenem	22	100
Tetracycline	16	72
Nitrofurantoin	15	68
Ofloxacin	19	86
Cotrimoxazole	15	68

[Table/Fig-3]: Antibiogram of carbapenem resistant *Klebsiella pneumoniae* (N=22).

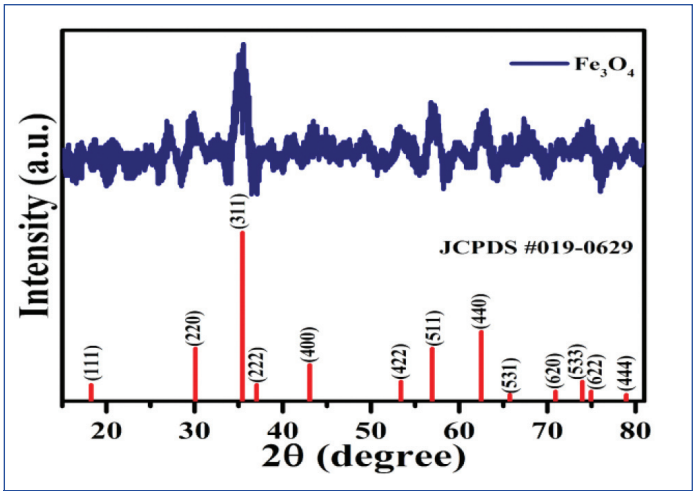
Antimicrobial agent	No. of resistant isolates	Resistance percentage (%)
Ampicillin	20	100
Amoxicillin clavulanate	19	95
Ceftazidime	20	100
Ceftazidime with clavulinic acid	20	100
Cefepime	20	100
Cefuroxime	20	100
Ceftriaxone	20	100
Ertapenem	20	100
Meropenem	19	95
Piperacillin tazobactum	20	100
Amikacin	9	45
Ciprofloxacin	20	100
Cefotaxime	20	100
Cefoxitin	20	100
Cefazolin	20	100
Gentamicin	12	60
Imipenem	20	100
Tetracycline	11	55
Nitrofurantoin	0	0
Ofloxacin	19	95
Cotrimoxazole	16	80

[Table/Fig-4]: Antibiogram of MDR *E. coli* (N=20).

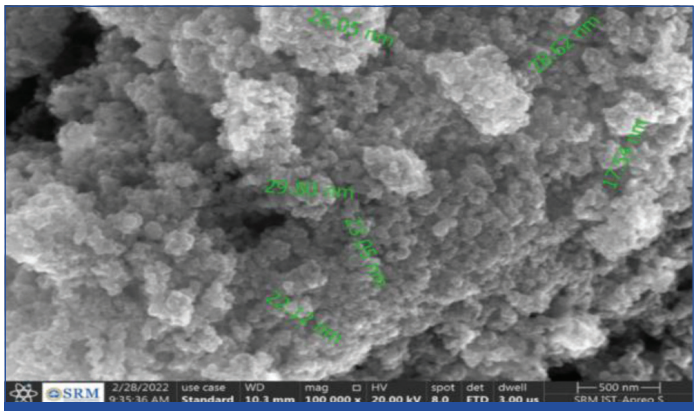
Scanning electron microscopy: The average size of the nanoparticle was identified as 24 nm [Table/Fig-6,7].

Charge characterisation of FeNp: Charge characterisation of FeNp was done using 2.5% AGE and the surface charge of prepared nanoparticles was identified as positive for bare iron oxide nanoparticles and negative for citrate coated (capped) iron oxide nanoparticles.

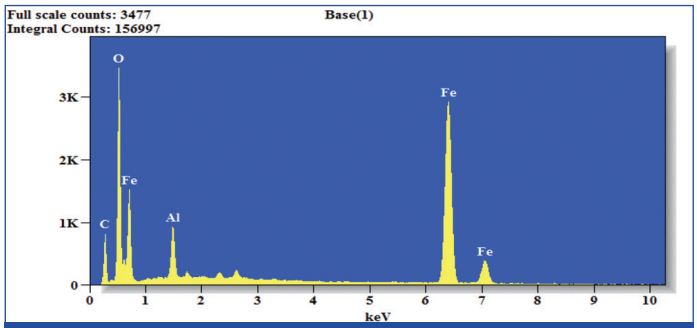
Minimum inhibitory concentration evaluation: Four different concentrations of bare and capped nanoparticles (0.0375 mg/mL,



[Table/Fig-5]: X-ray diffraction spectroscopy (Using BRUKER USA D8 Advance, Davinci crystallite size- 6.625 nm).



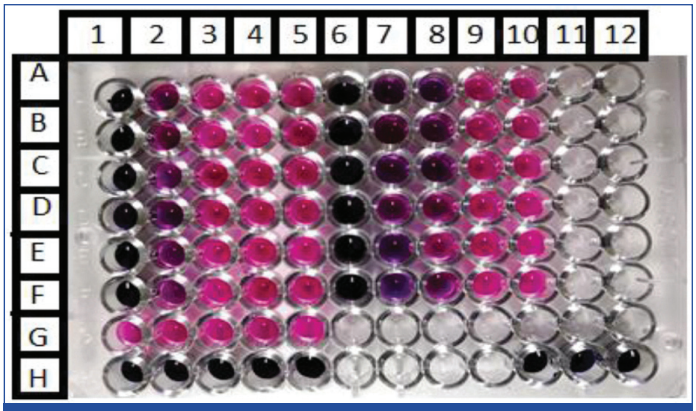
[Table/Fig-6]: Scanning electron microscopy (average size of nanoparticle 24 nm).



[Table/Fig-7]: Energy dispersive X-ray spectroscopy (analytical technique for elemental analysis.
The presence of other elements were observed as a result of use of aluminium foil during SEM analysis

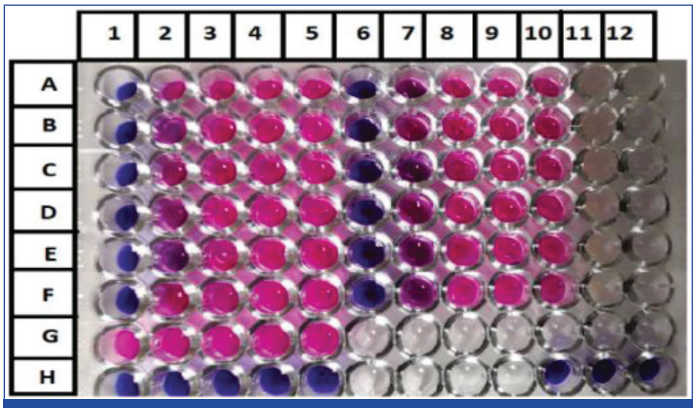
0.07 mg/mL, 0.15 mg/mL and 0.3 mg/mL) were used to determine the MIC value against MRSA isolates while 0.018 mg/mL, 0.0375 mg/mL, 0.07 mg/mL, and 0.15 mg/mL dilutions were used in case of CR *K. pneumoniae* and MDR *E. coli.*

MIC against Methicillin-resistant *Staphylococcus aureus* isolates (MRSA): The MIC determination of green synthesised Fe_3O_4 nanoparticles against MRSA isolates. Wells extending from columns 2 to 5 and row A to F and wells extending from columns 7 to 10 and row A to F contains different MRSA isolates with varying concentration of bare and capped nanoparticles respectively. Column 1 and 6 (row A to F) shows nanoparticles control with no organism. Columns 1 to 5 (row G) were positive controls that contain suspensions of bacterial isolates alone. Row H (column 1 to 5) and row H (column 10 to 12) were resazurin and citrate control respectively with no contamination. Since, colour change occurred in column 2 in the case of bare nanoparticles and column 8 in the case of capped nanoparticles, the MIC value is confirmed as 0.3 mg/mL for bare nanoparticles and 0.15 mg/mL for capped nanoparticles [Table/Fig-8].

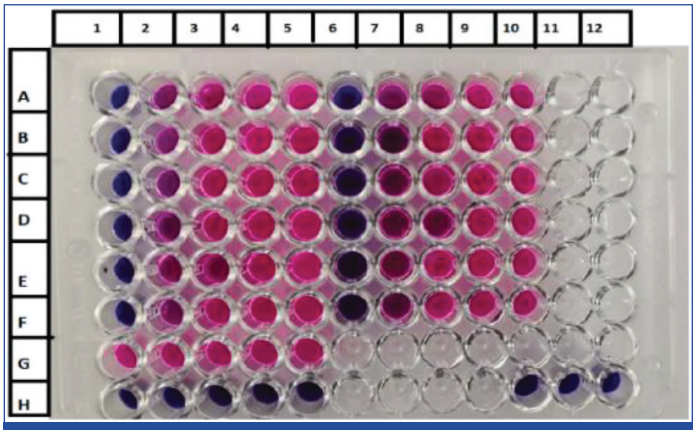


[Table/Fig-8]: MIC against MRSA.

MIC against MDR *E. coli* and CR *K. pneumoniae* isolates: Wells extending from columns 2 to 5 and row A to F and wells extending from columns 7 to 10 and row A to F contains isolates with varying concentration of bare and capped nanoparticles respectively. Column 1 and 6 (row A to F) shows nanoparticles control with no organism. Row G (columns 1 to 5) were positive controls that contains suspensions of bacterial isolates alone. Row H (column 1 to 5) and row H (column 10 to 12) were resazurin and citrate control respectively with no contamination. Since, colour change occurred in column 2 in the case of bare nanoparticles and column 7 in the case of capped nanoparticles, the MIC value is confirmed as 0.15 mg/mL for bare and capped nanoparticles in the case of both isolates [Table/Fig-9,10].



[Table/Fig-9]: MIC against CR *K. pneumoniae*.



[Table/Fig-10]: MIC against MDR *E. coli*.

DISCUSSION

Antibiotic resistance has become a hurdle that intimidates our ability to treat common infections. Heavy dose and combination therapies are the current treatment option against these pathogens. The rise in resistant pathogens against these combination drug therapies in the past decade has made them ineffective. A high dosage of the antibiotic does not turn out to be helpful in case of patients with co-morbidities and low immunity as seen in older individuals, so it is important to introduce novel techniques like

green nanotechnology to exploit the possibilities of overcoming this crisis.

Out of 50 urine samples, CR *K. pneumoniae* (22), MDR *E. coli* (20), and MRSA (8) were isolated. Since, MRSA pathogens are commonly associated with hospital-acquired infections, the number of isolates was comparatively less and most of them are from ICUs. Similar trends were reported by Sanjana RK et al., and Mallick SK and Basak S, in their studies conducted in Nepal and central India [14,15].

The maximum number of CR *K. pneumoniae* and MRSA was isolated from the age group above 60, were 63.5% and 62.5% respectively. However, in the case of MDR *E. coli*, the maximum number of isolates were from the age group 19 to 60 (65%). Lohan K et al., and Chellapandi K et al., found similar results in their studies [16,17].

In the case of MDR *E. coli* and CR *K. pneumoniae*, maximum isolates were from the General Medicine (GM) ward (30%). Kapoor G and Chaurasia D, in their study, revealed the same results of CR *K. pneumoniae* but it does not coincide with the results of MDR *E. coli* [18]. Since, the number of patients admitted in GM was considerably high, the chance of the spread of bacterial infection rises. The reasons behind CR *K. pneumoniae* and MRSA infection in ICU patients might be a result of prolonged hospitalisation, catheterisation, lack of surveillance and bundle approach.

While taking the antibiogram of MRSA into consideration, all eight isolates showed (100%) resistance to antibiotics such as penicillin, cefoxitin and bacitracin. A 50% of isolates were resistant to ciprofloxacin and 87% were resistant to erythromycin. All MRSA isolates were found sensitive to tetracycline, linezolid, chloramphenicol and nitrofurantoin. Variations in antibiogram patterns were reported in certain studies conducted by Lohan K et al., [16]. These variations might be due to the demographical changes pertaining to the geographical area and strains used.

However, the increasing resistance of *E. coli* and CR *K. pneumoniae* were in consideration CR *K. pneumoniae* showed resistance to most of the antibiotics and had 100% resistance to antibiotics of group penicillins, β lactams+β lactam inhibitors, 1st generation cephalosporins, carbapenems and aminoglycosides. Lowest resistance was observed against nitrofurans (68%), Sulphonamides (68%) and cefuroxime (68%). These results can be positively correlated with previous studies conducted by Indrajith S et al., [19]. Dissimilarity in resistance percentage might be a result of the change in the number of isolates.

Most of the MDR *E. coli* isolates were AmpC producers and carbapenem-resistant. They showed 100% resistance to penicillins, β lactam+β lactam inhibitors, 1st 2nd 3rd, and 4th generation cephalosporins, carbapenems except for meropenem (95%) and fluoroquinolones {(Ofloxacin (95%)}. Lowest resistance was observed against aminoglycosides {(Amikacin (45%) and gentamicin (60%)}, Tetracycline (55%) and nitrofurantoin (0%). The higher resistance to these antibiotics was reported in other study conducted by Nosheen S et al., in paediatric patients [20]. Contradictory results in few antibiotics were observed in a study conducted by Kibret M and Abera B [21]. Moreover, the difference in strains can be a reason for these results.

This study tries to utilise the ability of nanoparticles to disrupt the bacterial cell by the production of Reactive Oxygen Species (ROS), for considering the implementation of nanotechnology in antimicrobial therapy. The green synthesised Iron Oxide Nanoparticles (IONPs) and the citrate coated IONPs were characterised by FTIR spectrometry, XRD, SEM and UV-visible absorption spectra. As a result of XRD, 35.25° diffraction peaks are evident that accord with the crystal plane of (311) of crystalline Fe_3O_4 nanoparticles. The crystallite size of the magnetic Fe_3O_4 was found to be 6.625 nm. Studies conducted by Mahdavi N et al., corroborate these results [11]. In present study, during UV-visible absorption spectra the characteristic surface plasmon band is centered at 190-250 nm and the presence of nearby infrared absorption confirmed that the complex is Fe_3O_4. The presence of transient absorption bands between the wavelengths 360-320, 320-290, 290-270, and 270-250 nm also substantiated the formation of carbon functionalised nanoparticles. Four infrared bands (3319 cm^{-1}, 1641 cm^{-1}, 535 cm^{-1}, and 307 cm^{-1}) observed in FTIR in which signal at 3319 cm^{-1} corresponds to OH stretching. The charge of bare and surface modified nanoparticles was identified using AGE as positive and negative respectively. In 2016 Krohling CA et al., got similar results in their study of iron homeostasis [22].

Different concentrations of both bare and capped Fe_3O_4 nanoparticles were used to determine MIC against MRSA, MDR *E. coli*, and CR *K. pneumoniae*. MIC micro broth dilution using resazurin dye was used for this purpose. Out of the four dilutions, bare nanoparticles inhibited the growth of MRSA at 0.3 mg/mL while citrate coated nanoparticles inhibited the growth at 0.15 mg/mL. The reason behind this observation might be due to the better absorption of citrate coated nanoparticles as the MRSA are citrate utilising organisms. Production of siderophores by MRSA gives an added aid in Fe_3O_4 nanoparticles absorption [23]. Validating results were appreciated in other studies done by Cavassin ED et al., in the year 2015 [24].

Four different concentrations of bare and capped nanoparticles were tested with MDR *E. coli* and CR *K. pneumoniae* from which both organisms were inhibited at 0.15 mg/mL of bare and capped nanoparticles. The varying results while compared with MRSA might be because of the non utilisation of citrate by MDR *E. coli* and the presence of capsular proteins in *K. pneumoniae* [25,26]. Hence, this study revealed that green synthesised iron oxide nanoparticles have antibacterial activity against organisms such as MRSA, MDR *E. coli*, and CR *K. pneumoniae* isolated from urine samples of patients diagnosed with urinary tract infections. Considering that very few in-vitro and in-vivo studies have been conducted on the antimicrobial activity of citrate coated iron oxide nanoparticles, further research is needed to exploit nanotechnology to overcome antimicrobial resistance.

Limitation(s)

In-vitro studies have the limitation of laboratory and clinical setup errors. Further investigation on molecular mechanisms governing the antimicrobial activity of nanoparticles was not included in this study.

CONCLUSION(S)

Overuse and misuse of antibiotics have led to the spread of antimicrobial resistance globally and are on an unpredictable rise. The need for a novel lead molecule for the discovery and development of a new drug class is a high priority action to be undertaken in the field of microbiology and pharmacology. Adopting nanoparticle science as an aid to overcome this global hazard could bear fruitful results in the future. This study has successfully tapped into the possibility of utilising nanotechnology in the near future as perceptible susceptibility was detected for these pathogens towards iron oxide nanoparticles. Fe_3O_4 nanoparticles can act as a remarkable carrier for antibiotics and could be considered a serious contender in the development of new antibiotic molecules.

Acknowledgement

Sincere, thanks to Dr. KV Leela from SRM Medical College Hospital and Research Centre for their valuable guidance. Author thank Dr. Dalia Vishnudasan, Mr. Sujith R, Mr. Balamurali, Mr. Jayaprakash and Ms. Shubhangi Trisal, for their kind support throughout the project work. Authors acknowledge Sir C.V Raman Research Park and Amrita School of Biotechnology for the provision of resources and research amenities.

REFERENCES

[1] Loo YY, Rukayadi Y, Nor-Khaizura MAR, Kuan CH, Chieng BW, Nishibuchi M, et al. In-vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram negative foodborne pathogens. Front Microbiol. 2018;9:1555. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2018.01555.

[2] Wielders CLC, Fluit AC, Brisse S, Verhoef J, Schmitz FJ. *mecA* gene is widely disseminatedthe in *Staphylococcus aureus* population. J Clin Microbiol. 2002;40(11):3970-75. Available from: https://pubmed.ncbi.nlm.nih.gov/12409360.

[3] CLSI. Performance standards for antimicrobial susceptibility testing. 2021. M100.

[4] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2012;18(3):268-81.

[5] Band VI, Satola SW, Burd EM, Farley MM, Jacob JT, Weiss DS, et al. Carbapenem-resistant *Klebsiella pneumoniae* exhibiting clinically undetected colistin heteroresistance leads to treatment failure in a murine model of infection. M Bio. 2018;9(2):e02448-17. Available from: https://pubmed.ncbi.nlm.nih.gov/29511071.

[6] Herlekar M, Barve S, Kumar R. Plant-mediated green synthesis of Iron nanoparticles. Banerjee I, editor. J Nanoparticles. 2014;2014:140614. Available from: https://doi.org/10.1155/2014/140614.

[7] Loutfy H. Madkour. Nanoelectronic Materials Fundamentals and Applications. Springer Berlin Heidelberg. 2019.

[8] Saraswathy A, Nazeer SS, Jeevan M, Nimi N, Arumugam S, Vijayakumar S, et al. Citrate coated iron oxide nanoparticles with enhanced relaxivity for in-vivo magnetic resonance imaging of liver fibrosis. Colloids Surf B Biointerfaces. 2014;117:216-24. ISSN 0927-7765, https://doi.org/10.1016/j.colsurfb.2014.02.034.

[9] Alangari A, Alqahtani MS, Mateen A, Kalam MA, Alshememry A, Ali R, et al. Iron oxide nanoparticles: Preparation, characterization, and assessment of antimicrobial and anticancer activity. Adsorption Science and Technology. 2022;2022:01-09.

[10] Sreelakshmi B, Induja S, Adarsh PP, Rahul HL, Arya SM, Aswana S, et al. Drought stress amelioration in plants using green synthesised iron oxide nanoparticles. Mater Today Proc. 2020;41:723-27.

[11] Mahdavi M, Namvar F, Ahmad M Bin, Mohamad R. Green biosynthesis and characterization of magnetic iron oxide (Fe_3O_4) nanoparticles using seaweed (Sargassum muticum) aqueous extract. Molecules. 2013;18(5):5954-64. Available from: https://pubmed.ncbi.nlm.nih.gov/23698048.

[12] Rani GN, Budumuru R, Bandaru NR. Antimicrobial activity of honey with special reference to Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA). J Clin Diagn Res. 2017;11(8):DC05-08. Doi: 10.7860/JCDR/2017/30085.10347. Epub 2017 Aug 1. PMID: 28969119; PMCID: PMC5620759.

[13] Fattah RAFA, Fathy FEZY, Mohamed TAH, Elsayed MS. Effect of chitosan nanoparticles on quorum sensing-controlled virulence factors and expression of LasI and RhlI genes among *Pseudomonas aeruginosa* clinical isolates. AIMS Microbiol. 2021;7(4):415-30. Available from: https://pubmed.ncbi.nlm.nih.gov/35071940.

[14] Sanjana RK, Shah R, Chaudhary N, Singh YI. Prevalence and antimicrobial susceptibility pattern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in CMS-teaching hospital: A preliminary report. J Coll Med Sci. 2010;6(1):01-06.

[15] Mallick SK, Basak S. MRSA-too many hurdles to overcome: A study from Central India. Trop Doct. 2010;40(2):108-10. Available from: https://doi.org/10.1258/td.2010.090440.

[16] Lohan K, Sangwan J, Mane P, Lathwal S. Prevalence pattern of MRSA from a rural medical college of North India: A cause of concern. J Fam Med Prim Care. 2021;10(2):752-57.

[17] Chellapandi K, Dutta TK, Sharma I, De Mandal S, Kumar NS, Ralte L, et al. Prevalence of multidrug resistant enteropathogenic and enteroinvasive *Escherichia coli* isolated from children with and without diarrhea in Northeast Indian population. Ann Clin Microbiol Antimicrob . 2017;16(1):49. Available from: https://pubmed.ncbi.nlm.nih.gov/28693504.

[18] Kapoor G, Chaurasia D. Phenotypic prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing Enterobacteriaceae and antibiotic coresistance in a tertiary care hospital. Indian J Microbiol Res. 2017;4:90-95.

[19] Indrajith S, Mukhopadhyay AK, Chowdhury G, Farraj DA Al, Alkufeidy RM, Natesan S, et al. Molecular insights of carbapenem resistance *Klebsiella pneumoniae* isolates with focus on multidrug resistance from clinical samples. J Infect Public Health. 2021;14(1):131-38.

[20] Nosheen S, Bukhari NI, Ejaz H, Abbas N. Antibiogram and recent incidence of multidrug resistant carbapenemase producing *Escherichia coli* isolated from paediatric patients. Pakistan J Med Sci. 2020;36(2):246-50.

[21] Kibret M, Abera B. Antimicrobial susceptibility patterns of *E. coli* from clinical sources in northeast Ethiopia. Afr Health Sci. 2011;11(Suppl 1):S40-S45. Available from: https://pubmed.ncbi.nlm.nih.gov/22135643.

[22] Krohling CA, Eutrópio FJ, Bertolazi AA, Dobbss LB, Campostrini E, Dias T, et al. Ecophysiology of iron homeostasis in plants. Soil Sci Plant Nutr. 2016;62(1):39-47. Available from: https://doi.org/10.1080/00380768.2015.1123116.

[23] Perry WJ, Spraggins JM, Sheldon JR, Grunenwald CM, Heinrichs DE, Cassat JE, et al. *Staphylococcus aureus* exhibits heterogeneous siderophore production within the vertebrate host. Proc Natl Acad Sci U S A. 2019;116(44):21980-82.

[24] Cavassin ED, de Figueiredo LFP, Otoch JP, Seckler MM, de Oliveira RA, Franco FF, et al. Comparison of methods to detect the in-vitro activity of silver nanoparticles (AgNP) against multidrug resistant bacteria. J Nanobiotechnology. 2015;13(1):64. Available from: https://doi.org/10.1186/s12951-015-0120-6.

[25] Campos MA, Vargas MA, Regueiro V, Llompart CM, Albertí S, Bengoechea JA, et al. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun. 2004;72(12):7107-14.

[26] Moini AS, Soltani B, Taghavi Ardakani A, Moravveji A, Erami M, Haji Rezaei M, et al. Multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolated from patients in Kashan, Iran. Jundishapur J Microbiol. 2015;8(10):e27517.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, Chengalpattu, Tamil Nadu, India.
2. Assistant Professor, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, Chengalpattu, Tamil Nadu, India.
3. Postgraduate, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, Chengalpattu, Tamil Nadu, India.
4. Professor and Head, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, Chengalpattu, Tamil Nadu, India.
5. Assistant Professor, Department of Biotechnology, Amrita School of Biotechnology, Amritavishwa Vidyapeetham, Amritapuri, Clappana, Kollam, Kerala, India.
6. Research Scholar, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, Chengalpattu, Tamil Nadu, India.
7. Research Scholar, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, Chengalpattu, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Balamurali Venkatesan,
Research Scholar, Department of Microbiology, SRM Medical College Hospital and Research Centre, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, Chengalpattu, Tamil Nadu, India.
E-mail: balajai96@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jun 01, 2022
- Manual Googling: Aug 13, 2022
- iThenticate Software: Aug 22, 2022 (6%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **May 26, 2022**
Date of Peer Review: **Jul 01, 2022**
Date of Acceptance: **Aug 24, 2022**
Date of Publishing: **Oct 01, 2022**