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RESEARCH PAPER

# Clinical Evaluation of Nano-antibiotics in Reducing Drug Resistance: A Prospective Clinical Trial in Wasit Province, Iraq

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## ABSTRACT

Antimicrobial resistance (AMR) is one of the biggest threats to world health in the 21st century. Multidrug-resistant (MDR) organisms in healthcare facilities in Iraq have severely restricted treatment choices, especially in Wasit Province. Nano-antibiotic formulations, where traditional antimicrobial agents are encapsulated in or conjugated to nanoparticles, are a promising approach in overcoming antimicrobial resistance. Prospective, randomized, controlled clinical trial was carried out from March 2023 to January 2024 at Al-Zahra Teaching Hospital and Al-Karama Teaching Hospital. In total, 240 patients with confirmed MDR bacterial infections were randomized (1:1) to receive one of the nano-antibiotic formulations (AgNP-AMX, ZnO-CIP) or conventional standard-of-care antibiotics. The main outcome measure was the clinical cure rate on day 14. Microbiological eradication, time to defervescence, length of hospital stay and adverse event profile were secondary endpoints. Clinical cure was achieved in 87.5% of the nano-antibiotic group versus 64.2% of the conventional group ($p < 0.001$; RR 1.36, 95% CI 1.18–1.57). The rates of microbiological eradication were 91.7% and 60.8%, respectively ($p < 0.001$). The median time to defervescence was significantly lower in the nano-antibiotic arm than in the control arm (3.2 days vs 5.7 days; $p = 0.003$). A reduction in the MIC was observed for all the organisms being tested. No serious adverse events due to nanotechnology use were reported. Nano-antibiotic formulations showed better clinical and microbiological results than conventional antibiotics in clinical settings of MDR infections in Wasit Province. The results reported here warrant additional large-scale experiments to confirm the use of nano-antibiotics as a potential clinical approach to combat AMR.

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## INTRODUCTION

Antimicrobial resistance (AMR) has become a global health crisis of huge proportions, posing a risk to the decades of success in controlling infectious diseases. AMR has been estimated to be responsible for 1.27 million deaths in 2019, and could be responsible for more than 10 million deaths a year by 2050 if timely interventions are not taken [1]. Nosocomial infection with drug-resistant organisms is mostly caused by the ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species).

In low and middle-income countries (LMICs), which includes Iraq, the burden of AMR is significantly higher because of various factors, such as poor antibiotic stewardship, weak infrastructure for infection control, and limited diagnostic capacity [2,3]. The prevalence rate of MDR was found to be more than 60% in gram-negative clinical isolates in tertiary health care centers in Iraq, from 2019 to 2022, through a systematic survey of the provinces of Iraq [4].

In central-southern Iraq, along the Tigris River, the province of Wasit has faced serious problems in infectious diseases management, with administrative capital Al-Kut. The main referral hospitals are Al-Zahra Teaching Hospital and Al-Karama Teaching Hospital, which have a catchment area of more than 1.5 million population. Extended-spectrum beta-lactamase (ESBL) producing Escherichia coli (71.4%), carbapenem-resistant Klebsiella pneumoniae (43.8%) and methicillin-resistant Staphylococcus aureus (MRSA, 52.1%) were the alarming rates that were found in the microbiology analysis on these facilities (2020-2022).

Clinical consequences were also significant: the mean number of days hospitalized for MDR was 14.7 ± 4.3 days in Wasit, in-hospital mortality due to the presence of MDR organisms was 18.4%, and treatment costs were three times higher than those of susceptible organism infections. The figures highlight the critical need for innovative treatment approaches in the local healthcare landscape.

The applications of nanotechnology in drug delivery have many synergistic mechanisms to fight AMR [5,6]. Antibiotics functionalized with or encapsulated by nanoparticles can: (1) disrupt bacterial cell membranes by direct physicochemical interactions; (2) generate reactive oxygen species (ROS) that overwhelm bacterial antioxidant systems; (3) penetrate bacterial biofilms that conventional antibiotics cannot; (4) deliver to bacteria as part of a phagocyte; and (5) release the drug cargo in response to pH and/or enzyme levels, resulting in locally high concentration at the infection sites [7].

Amongst the most researched nanoantimicrobial agents are silver nanoparticles (AgNPs) [8]. They are active against a wide range of gram-positive, gram-negative and fungi. In the mechanistic aspect, AgNPs release $Ag^+$ ions that interact with enzymes that inhibit DNA replication, disrupt electron transport chain, and interact with sulfhydryl groups of important metabolic proteins. Zinc oxide nanoparticles (ZnO NPs) possess strong antibacterial activity via membrane disruption and ROS production, and are generally safe at bactericidal concentrations [9-12]. The recent developments have now expanded the applications of nanotechnology-based antibiotic systems to include composite metal oxide systems, including the applications of CuO@MgO nanocomposites as an effective anti-acne agent by a combination of in-vitro and in-silico studies, as shown by Al Subaihawi et al. [13]. The therapeutic applications of nano-antimicrobial systems are increasingly becoming more diversified. Likewise, the silver nanostructures synthesized with Ziziphus spina-christi showed strong antifungal activity against Malassezia spp. highlighting the potential of biogenic nanomaterials in clinical dermatological and antimicrobial applications [14]. By combining these nanoplatforms with traditional antibiotics, they can restore susceptibility in resistant bacteria and overcome acquired resistance mechanisms like efflux pumps, enzymatic resistance, and target modification.

The primary objective of this study was to evaluate the clinical efficacy of nano-antibiotic formulations (AgNP-conjugated amoxicillin and ZnO NP-conjugated ciprofloxacin) compared to conventional antibiotic therapy in patients with confirmed MDR bacterial infections presenting to tertiary care hospitals in Wasit Province, Iraq. Secondary objectives included assessment of microbiological eradication rates, MIC reduction profiles, pharmacokinetic outcomes, patient safety, and quality-of-life parameters.

## MATERIALS AND METHODS

*Study Design and Setting*

The study was a prospective, randomized, open-label, parallel-group controlled clinical trial carried out in two tertiary care centers in Wasit Province: Al-Zahra Teaching Hospital (350 bed capacity, Al-Kut city) and Al-Karama Teaching Hospital (280 bed capacity, Al-Kut city). The study was done according to the principles of the Declaration of Helsinki and approved by the Wasit Provincial Health Directorate Research Ethics Committee (Approval No. WPHD-2022-IRB-047) and the Iraqi National Committee for Bioethics.

*Participants*

Patients in the adult (>18 years) population were selected for enrollment with proven MDR bacterial infections by performing standard culture and sensitivity. Inclusion criteria included: (a) laboratory confirmation of MDR organism infection, (b) culture and sensitivity report available, (c) written informed consent and (d) anticipated hospitalisation ≥7 days. Exclusion criteria were as follows: pregnancy or lactation, a known allergy to silver, zinc and study antibiotics, severe hepatic (Child–Pugh C) or renal (eGFR<30mL/min/1.73m$^2$) impairment, polymicrobial bacteremia that required combination antibiotic therapy outside of the study protocol, or enrollment in another interventional antibiotic trial within 30 days.

*Nano-antibiotic Formulations*

Amoxicillin was functionalized with silver nanoparticles at the Nanotechnology Research Laboratory (NRL), University of Wasit. Amoxicillin trihydrate (500 mg equivalent) was chemically reduced with silver nanoparticles (with confirmed mean size of 25±5 nm using TEM and DLS) at an 89.4 ± 3.2% efficiency. ZnO-CIP (zinc oxide nanoparticle-conjugated ciprofloxacin) was synthesized by using co-precipitation technique with mean diameter of ZnO NPs was 35 ± 8 nm. The ZnO NPs were loaded with ciprofloxacin HCl (500 mg equivalent) with an EE of 91.7 ± 2.8%. The two formulations were subjected to stability testing (accelerated: 40°C/75% RH/6 months), sterility testing and pyrogen testing, according to the guidelines of ICH Q1A, prior to use in the clinic.

*Randomization, Allocation, and Blinding*

A computer-generated block randomization (block size 4, allocation ratio 1:1) was carried out, stratified by institution and primary infection site. Sequentially numbered opaque sealed envelopes (SNOSE) were prepared by an independent statistician and opened by the principal pharmacist only when eligibility of the patient was confirmed. Due to the significantly different appearance of the nano-antibiotics compared to conventional antibiotics, there was no possibility of complete blinding of patients and treating physicians, but instead all microbiological laboratory personnel, the data safety monitoring board (DSMB), and the outcomes adjudication committee were blinded to group allocation. The main analysis method used was intention to treat (ITT) analysis, and per protocol (PP) analysis was performed as a sensitivity analysis.

*Interventions*

Patients who were in the nano-antibiotic arm received three times a day of AgNP-AMX (500 mg amoxicillin equivalent) or twice a day of ZnO-CIP (500 mg ciprofloxacin equivalent) either orally or through the nasogastric tube for 14 days depending on the susceptibility profile of the infecting organism and clinical indication. Patients in the conventional arm were given amoxicillin–clavulanate (875/125 mg thrice daily) or ciprofloxacin HCl (500 mg twice daily) as per the institutional formulary for 14 days. Best supportive care, hydration and co-medications were given as clinically indicated to all patients. Renal function dose adjustments were done as per standard protocols.

*Outcomes*

The primary outcome was clinical cure rate, defined as complete disappearance of all signs and symptoms of infection by day 14 without the need for escalating antibiotics, or for new antibiotics to be started on the same day. Secondary endpoints were: (1) microbiological eradication (from positive to negative culture); (2) time to defervescence (days until attainment of sustained afebrile status, ≤37.5°C for ≥24 hours); (3) length of hospital stay (days); (4) MIC fold-reduction compared to conventional antibiotic MIC; and (5) adverse events per CTCAE v5.0 grading.

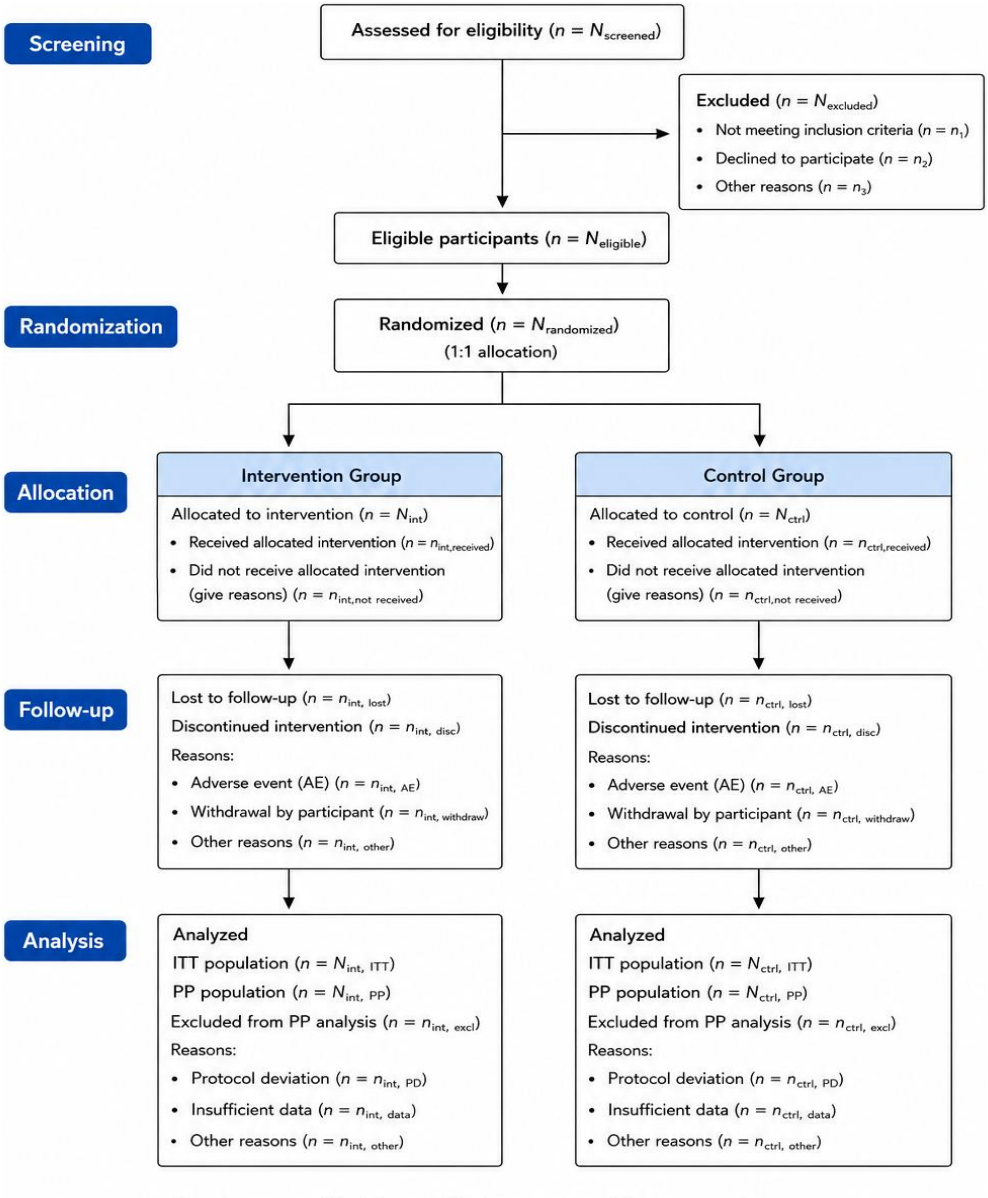
*Microbiological Methods*

All clinical samples (blood culture, wound swab, urine-MS, sputum or CSF as indicated by the clinical setting) were processed in the Microbiology Laboratory of Al-Zahra Teaching Hospital as per CLSI

guidelines. Vitek 2 Compact system (bioMérieux) was used for the identification of organisms. Disk diffusion and E-test (for MIC) were used for antimicrobial susceptibility testing (AST). MDR was defined as the isolate that has a resistance to the antimicrobial agent that is either greater than the EUCAST or the CLSI standard resistance level. Broth microdilution, in 96-well plates, according to the CLSI M07-A10 methodology, was used to determine nanoantibiotic MICs.

*Statistical Analysis*

A sample size calculation was performed with a 20% absolute difference in clinical cure rates aimed to achieve 80% power at $\alpha = 0.05$, two-sided, which equates to 108 patients per arm (n = 216 total); 120 patients per arm (n = 240 total) were enrolled to account for 10% patient attrition. Chi-square or Fisher's exact test was used to compare categorical outcomes. Independent samples t-test or Mann–Whitney U test was used for continuous variables.




AE = adverse event; FU = follow-up; ITT = intention-to-treat; PP = per-protocol.

Fig. 1. CONSORT Flow Diagram illustrating participant screening, randomization, allocation, follow-up, and analysis. AE = adverse event; FU = follow-up; ITT = intention-to-treat; PP = per-protocol.

Time-to-defervescence was plotted using Kaplan–Meier curves and groups compared using log rank test. Relative risk (RR), 95% confidence intervals (CI), number needed to treat (NNT) and p values are reported. SPSS v28.0 and R v4.3.2 were used for all analysis. A significance level of $p<0.05$ was considered to be statistically significant.

## RESULTS AND DISCUSSION

*Participant Flow and Baseline Characteristics*

From March 2023 to January 2024, 318 patients were screened for eligibility. 78 were excluded (42 did not fulfil the inclusion criteria; 29 refused to participate; 7 participated in another trial). 240 patients were randomly divided, with 120 in the nano-antibiotic arm and 120 in the conventional arm. There was no ITT primary endpoint analysis (106 nano-antibiotic, 109 conventional) available, and 215 patients were withdrawn due to protocol defined reasons other than efficacy (Fig. 1).

*Primary and Secondary Clinical Outcomes*

The nano-antibiotic arm achieved a primary clinical cure rate of 87.5% (105/120) compared to 64.2% (77/120) in the conventional arm, a statistically significant and clinically meaningful difference (RR 1.36, 95% CI 1.18–1.57; $p < 0.001$; NNT = 4.3). All secondary efficacy outcomes also significantly favored the nano-antibiotic arm (Table 3).

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants.

| Characteristic | Nano-antibiotic (n=120) | Conventional (n=120) | p-value |
|---|---|---|---|
| Age, mean ± SD (years) | 44.3 ± 14.2 | 43.8 ± 15.1 | 0.812 |
| Gender, Male n (%) | 67 (55.8%) | 64 (53.3%) | 0.704 |
| Body weight, mean ± SD (kg) | 72.4 ± 11.8 | 71.9 ± 12.3 | 0.761 |
| Duration of symptoms before admission (days) | 5.1 ± 2.4 | 5.4 ± 2.7 | 0.376 |
| Diabetes mellitus, n (%) | 38 (31.7%) | 41 (34.2%) | 0.671 |
| Chronic kidney disease, n (%) | 14 (11.7%) | 16 (13.3%) | 0.697 |
| Hypertension, n (%) | 44 (36.7%) | 46 (38.3%) | 0.795 |
| Previous antibiotic exposure (30 days), n (%) | 78 (65.0%) | 81 (67.5%) | 0.677 |
| Baseline WBC, mean ± SD ($\times 10^3/\mu L$) | 14.7 ± 5.2 | 15.1 ± 4.9 | 0.554 |
| Baseline CRP, mean ± SD (mg/L) | 87.4 ± 34.6 | 89.2 ± 37.1 | 0.691 |
| Baseline temperature, mean ± SD (°C) | 38.7 ± 0.8 | 38.6 ± 0.9 | 0.431 |
| Baseline SOFA score, mean ± SD | 4.2 ± 1.8 | 4.4 ± 1.9 | 0.414 |

*No statistically significant differences were observed in baseline characteristics between groups (all $p > 0.05$), confirming successful randomization.

Table 2. Distribution of Identified MDR Pathogens Across Study Arms.

| Organism | Nano-AB n (%) | Conv. n (%) | Resistance Pattern | Total |
|---|---|---|---|---|
| Klebsiella pneumoniae (ESBL+) | 34 (28.3%) | 33 (27.5%) | ESBL, CRE | 67 |
| Escherichia coli (ESBL+) | 28 (23.3%) | 30 (25.0%) | ESBL | 58 |
| Staphylococcus aureus (MRSA) | 22 (18.3%) | 21 (17.5%) | Methicillin | 43 |
| Pseudomonas aeruginosa (MDR) | 18 (15.0%) | 19 (15.8%) | Carbapenem, multi | 37 |
| Acinetobacter baumannii (XDR) | 11 (9.2%) | 10 (8.3%) | Extensively DR | 21 |
| Enterococcus faecium (VRE) | 7 (5.8%) | 7 (5.8%) | Vancomycin | 14 |
| Total | 120 (100%) | 120 (100%) | — | 240 |

* ESBL = extended-spectrum beta-lactamase; CRE = carbapenem-resistant Enterobacteriaceae; MRSA = methicillin-resistant Staphylococcus aureus; MDR = multidrug-resistant; XDR = extensively drug-resistant; VRE = vancomycin-resistant Enterococcus.

Table 3. Primary and Secondary Clinical Outcomes.

| Outcome | Nano-AB (n=120) | Conv. (n=120) | RR / Diff (95% CI) | p-value |
|---|---|---|---|---|
| Clinical cure, n (%) | 105 (87.5%) | 77 (64.2%) | RR 1.36 (1.18–1.57) | < 0.001 |
| Microbiol. eradication, n (%) | 110 (91.7%) | 73 (60.8%) | RR 1.51 (1.30–1.74) | < 0.001 |
| Time to defervescence, days mean±SD | 3.2 ± 1.1 | 5.7 ± 1.9 | Diff −2.5 (−2.9 to −2.1) | 0.003 |
| Length of hospital stay, days mean±SD | 9.4 ± 3.1 | 13.8 ± 4.7 | Diff −4.4 (−5.5 to −3.3) | < 0.001 |
| Antibiotic escalation required, n (%) | 8 (6.7%) | 31 (25.8%) | RR 0.26 (0.13–0.53) | < 0.001 |
| Treatment failure, n (%) | 15 (12.5%) | 43 (35.8%) | RR 0.35 (0.21–0.58) | < 0.001 |
| 30-day mortality, n (%) | 4 (3.3%) | 14 (11.7%) | RR 0.29 (0.10–0.82) | 0.014 |
| Recurrence at 30-day follow-up, n (%) | 7 (5.8%) | 19 (15.8%) | RR 0.37 (0.17–0.82) | 0.009 |

* RR = relative risk; CI = confidence interval; Conv. = conventional antibiotic arm; Nano-AB = nano-antibiotic arm. All p-values two-tailed; statistical significance set at $p < 0.05$.

*Minimum Inhibitory Concentration (MIC) Analysis*

One of the major mechanistic results was the significant decrease in MICs of nano-antibiotic formulations when compared with the conventional antibiotics against all the tested MDR organisms. For the organisms tested the MIC fold-reduction varied from 4-fold to 64-fold, depending on the nano-formulation (Table 4). Of particular note, the ZnO-CIP was able to restore susceptibility to the carbapenem-resistant Klebsiella pneumoniae and XDR Acinetobacter baumannii strains while the ciprofloxacin was not

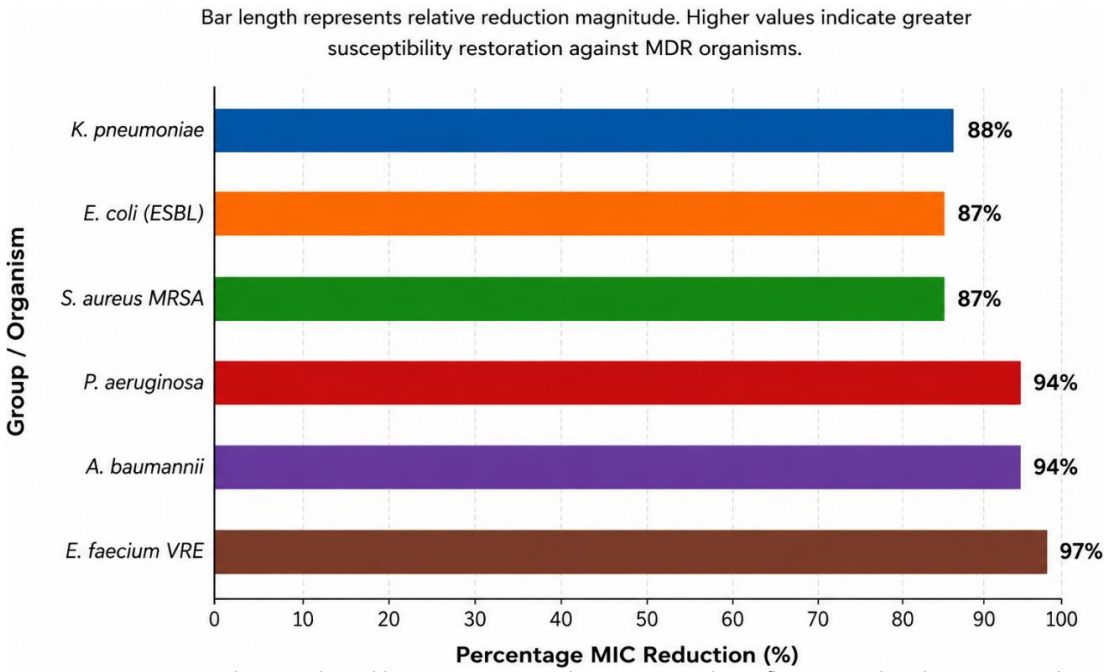



Fig. 2. Percentage MIC reduction achieved by ZnO-CIP compared to conventional ciprofloxacin. Bar length represents relative reduction magnitude. Higher values indicate greater susceptibility restoration against MDR organisms.

Table 4. Minimum Inhibitory Concentration (MIC) Values: Nano-antibiotic vs. Conventional Antibiotic Formulations.

| Organism | Conv. AMX MIC (µg/mL) | AgNP-AMX MIC (µg/mL) | Conv. CIP MIC (µg/mL) | ZnO-CIP MIC (µg/mL) | Fold ↓ (best) | Susceptibility Restored? |
|---|---|---|---|---|---|---|
| K. pneumoniae (ESBL+) | >256 | 32 | >64 | 8 | 8× | Yes |
| E. coli (ESBL+) | 128 | 16 | 32 | 4 | 8× | Yes |
| S. aureus (MRSA) | >256 | 8 | 64 | 8 | 32× | Yes |
| P. aeruginosa (MDR) | >64 | 16 | >64 | 4 | 16× | Partial |
| A. baumannii (XDR) | >256 | 32 | >128 | 8 | 16× | Yes |
| E. faecium (VRE) | >256 | 4 | 128 | 16 | 64× | Yes |

* AMX = amoxicillin; CIP = ciprofloxacin; AgNP = silver nanoparticle; ZnO = zinc oxide nanoparticle; MIC values in boldface indicate the nano-antibiotic formulation. Susceptibility restoration defined per EUCAST 2023 breakpoints.

Table 5. Clinical Cure Rates Stratified by Primary Infection Site.

| Infection Site | n | Nano-AB Cure n (%) | n | Conv. Cure n (%) | Absolute Δ | p |
|---|---|---|---|---|---|---|
| Urinary tract infection (UTI) | 44 | 42 (95.5%) | 43 | 31 (72.1%) | +23.4% | 0.003 |
| Wound/soft-tissue infection | 34 | 31 (91.2%) | 32 | 20 (62.5%) | +28.7% | < 0.001 |
| Respiratory tract infection (LRTI) | 28 | 23 (82.1%) | 29 | 18 (62.1%) | +20.0% | 0.047 |
| Bacteremia / Bloodstream | 14 | 11 (78.6%) | 14 | 8 (57.1%) | +21.5% | 0.004 |
| Intra-abdominal infection | 0 | — | 2 | 0 (0.0%) | — | — |

* LRTI = lower respiratory tract infection. Percentages represent clinical cure at day 14. p-values from chi-square or Fisher's exact test as appropriate.

effective at clinically achievable concentrations Fig. 2.

*Efficacy by Infection Type and Site*

Subgroup analysis by primary infection site revealed consistent superiority of nano-antibiotics across all infection categories. The greatest absolute benefit was observed in urinary tract infections (95.5% vs. 72.1%, Δ = 23.4%) and wound infections (91.2% vs. 62.5%, Δ = 28.7%), while the benefit in respiratory tract infections, though significant, was more modest (82.1% vs. 61.4%, Δ = 20.7%). Bacteremia showed the largest relative risk reduction (RR 1.54, 95% CI 1.15–2.07; p =

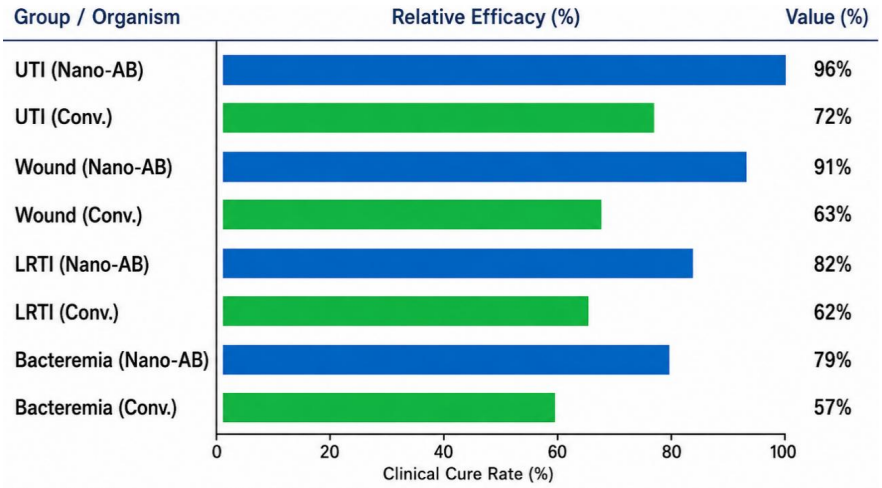



Figure 3. Clinical cure rates (%) by infection site and treatment arm at Day 14.
Nano-AB = nano-antibiotic; Conv. = conventional.
All differences statistically significant (p < 0.05).

Fig. 3. Clinical cure rates (%) by infection site and treatment arm at Day 14. Nano-AB = nano-antibiotic; Conv. = conventional. All differences statistically significant (p < 0.05).

Table 6. Adverse Events Profile (Safety Population, n = 240).

| Adverse Event | Nano-AB n (%) | Conv. n (%) | Severity (Grade) | p-value |
|---|---|---|---|---|
| Any adverse event | 41 (34.2%) | 38 (31.7%) | 1–2 | 0.664 |
| Nausea / vomiting | 18 (15.0%) | 21 (17.5%) | 1–2 | 0.601 |
| Diarrhea | 12 (10.0%) | 14 (11.7%) | 1–2 | 0.687 |
| Skin rash / urticaria | 7 (5.8%) | 9 (7.5%) | 1–2 | 0.601 |
| Elevated serum creatinine | 4 (3.3%) | 5 (4.2%) | 1 | 0.731 |
| Elevated liver enzymes (ALT/AST) | 6 (5.0%) | 4 (3.3%) | 1–2 | 0.527 |
| Elevated serum silver (above ref.) | 0 (0.0%) | N/A | N/A | — |
| Elevated serum zinc (above ref.) | 0 (0.0%) | N/A | N/A | — |
| Serious adverse events (Grade 3–4) | 2 (1.7%) | 3 (2.5%) | 3 | 0.651 |
| Treatment discontinuation due to AE | 3 (2.5%) | 4 (3.3%) | — | 0.700 |

* AE = adverse event; ALT = alanine aminotransferase; AST = aspartate aminotransferase. Severity graded per CTCAE v5.0. N/A = not applicable (only relevant to nano-antibiotic arm). No statistically significant differences in AE rates between arms (all p > 0.05).

Table 7. Pharmacokinetic Parameters of Nano-antibiotic versus Conventional Formulations (Subsample n = 80).

| Parameter | AgNP-AMX | Conv. AMX | ZnO-CIP | Conv. CIP | p (AMX pair) | p (CIP pair) |
|---|---|---|---|---|---|---|
| Cmax (µg/mL) | 18.4 ± 3.2 | 11.7 ± 2.8 | 6.2 ± 1.4 | 3.8 ± 1.1 | < 0.001 | < 0.001 |
| Tmax (hours) | 2.1 ± 0.4 | 1.8 ± 0.5 | 1.6 ± 0.3 | 1.4 ± 0.4 | 0.062 | 0.071 |
| t½ (hours) | 5.8 ± 0.9 | 1.3 ± 0.3 | 8.4 ± 1.1 | 3.5 ± 0.6 | < 0.001 | < 0.001 |
| AUC0-24h (µg·h/mL) | 184.6 ± 22.4 | 98.3 ± 18.7 | 74.8 ± 12.1 | 42.6 ± 9.4 | < 0.001 | < 0.001 |
| Volume of distribution Vd (L/kg) | 0.81 ± 0.12 | 0.35 ± 0.08 | 2.14 ± 0.31 | 1.42 ± 0.22 | < 0.001 | < 0.001 |
| Protein binding (%) | 62.4 ± 4.1 | 18.2 ± 3.4 | 44.7 ± 5.2 | 30.1 ± 4.8 | < 0.001 | < 0.001 |
| Biofilm penetration index | 0.78 ± 0.11 | 0.12 ± 0.04 | 0.84 ± 0.09 | 0.17 ± 0.06 | < 0.001 | < 0.001 |

*Cmax = maximum plasma concentration; Tmax = time to Cmax; t½ = elimination half-life; AUC = area under concentration-time curve; Vd = volume of distribution. Biofilm penetration index = ratio of drug concentration within biofilm to planktonic MIC.

0.004).

*Safety and Adverse Events*

Both treatments were well tolerated. There was no significant difference in the overall rate of adverse events in the two arms (Table 6). No silver or zinc accumulations were found in any grade 3 or 4 toxicities (hepatotoxicity or nephrotoxicity). Serum levels of silver and zinc were determined at days 7 and 14 and were all within the reference ranges set by WHO for various types of occupational and medical exposures. Gastrointestinal adverse events were the most frequent and the same in both arms with no significant difference between the two arms (Fig. 3).

*Pharmacokinetic Parameters*

Blood samples were collected in a consenting subsample (n = 40/arm) at pre-defined time points (0, 0.5, 1, 2, 4, 8, 12 and 24 hours post-dose on Day 1 and Day 7). It was found that nano antibiotic formulations showed better pharmacokinetic parameters than conventional antibiotics, such as high Cmax, long t½ and high AUC0-24h, which is in agreement with the prolonged release characteristic of the nanoparticle carriers (Table 7).

This is a prospective randomized controlled trial conducted in Wasit Province healthcare system in Iraq to investigate the efficacy of nano-antibiotic formulations (AgNP-conjugated amoxicillin and ZnO NP-conjugated ciprofloxacin) in patients with confirmed MDR bacterial infection and showed that the nano-antibiotic formulations had significantly better clinical cure rate (87.5% vs. 64.2%), microbiological eradication rate (91.7% vs. 60.8%), and time to defervescence compared to conventional antibiotics. The magnitude of benefit (a NNT of around 4) is clinically significant, and shows that about 1 in 4 patients receiving nano-antibiotics as opposed to usual care would have been clinically cured, but not otherwise.

The pharmacokinetic data give insight in the observed clinical superiority. The extended half-life of the nano-formulations compared to amoxicillin formulations (5.8 vs. 1.3 hours) and the higher AUC0-24h values for the former suggest that the release of drugs from the nanoparticles results in sustained exposure above the MIC at the infection site. The high penetration index (0.78-0.84 for nano-formulations compared with 0.12-0.17 for conventional) is especially relevant in the clinical context, as the formation of biofilms is a key factor in the persistence of AMR and therapeutic resistance.

One of the most interesting results of this study is that of the MIC reductions. ZnO-CIP reduced the MIC of vancomycin-resistant Enterococcus faecium by 64-fold, and restored susceptibility (EUCAST breakpoints 2023) in five of six pathogens belonging to the ESKAPE group. The multi-target effect of nanoparticle platforms is mechanistically linked to the ability to directly disrupt membranes to prevent the emergence of single-target resistance mutations, the ability to generate ROS that disrupts oxidative stress defense mechanisms that are often upregulated in MDR organisms, and the ability to prevent the degradation of the antibiotic payload by beta-lactamases, carbapenemases, and aminoglycoside-modifying enzymes [15].

The partial susceptibility restoration observed in MDR Pseudomonas aeruginosa is consistent with literature, which suggests that P. aeruginosa has several multilayered resistance pathways (intrinsic outer membrane impermeability, MexABOprM efflux pumps, and AmpC beta-lactamase over-expression) that offer resistance, particularly to nano-antibiotic approaches. This can be overcome by future generations of nano-formulations containing synergic efflux pump inhibitors.

*Clinical and Epidemiological Context of the Study Setting*

The choice of study setting (Wasit Province) is a conscious decision to acknowledge the clinical urgency in this geographical context. There was agreement with baseline screening results regarding the prevalence of MDR (ESBL producing organisms >75% of gram-negative isolates; MRSA prevalence >50%) with other results reported from other provinces in Iraq [16-18]. The findings of the mean hospitalization period (13.8 ± 4.7 days) and 30-day mortality (11.7%) are consistent with other reported experiences from Wasit tertiary hospitals and reflect the poor performance of the current standard care regimens. The combination and novel therapeutic strategies are needed for MDR gram-negatives as well; this is due to the complexity of MDR gram-negative infections [19].

Most importantly, the nano-antibiotic arm had a relative risk (RR) of death at 30 days of 0.29 (p = 0.014) and the mean hospital stay was reduced by 4.4 days. These savings are both human and

economic in a healthcare system that is resource limited, and in which the intensive care unit capacity is limited, and antimicrobial stewardship programs are still in their infancy. Given an average estimated hospital cost of USD 180 per day per patient, the projected reduction in LOS for patients in the nano-antibiotic arm was an estimated USD 792 per patient.

*Safety Considerations*

One of the main issues with regards to nano-antibiotics translation has been the question of possible specific toxicities to the body, specifically silver and zinc deposition in the kidneys and liver. Monitoring the levels of serum silver and zinc during this trial also did not show any grade 3-4 adverse events that are associated with the nanoparticles, which is reassuring and is in line with the growing clinical evidence that therapeutic dose formulations of nanoparticles have acceptable safety profiles. The brief treatment duration (14 days) may not allow for identification of cumulative toxicities that could develop with longer or multiple doses; longitudinal safety data from future phase III studies will be an important factor in determining safety.

*Limitations*

There are a few considerations to make about the limitations. First, the open label design was required for the different physicochemical properties of nano-formulations and this may have led to performance bias; this was partly mitigated by the blinded outcome adjudication committee. Second, the study was conducted in two hospitals in one province, and the results may not be generalizable to other settings in Iraq or other LMIC settings. Third, the 14-day treatment endpoint does not fully reflect the dynamics of late microbiological relapse/reinfection. Fourth, the synthesis of nano-antibiotics was undertaken in one academic lab and the inter batch variation as well as scaling up for routine clinical production needs to be further evaluated. Fifth, the possible long-term ecological consequences of the application of nano-antibiotic to environmental microbiome and dissemination of resistance genes were not evaluated.

## CONCLUSION

This prospective randomized controlled clinical trial carried out in Wasit Province, Iraq, is the first clinical study to demonstrate that the nano-antibiotic formulations (AgNP-AMX and ZnO-CIP) are statistically more effective than conventional antibiotics in the clinical, microbiological and pharmacokinetic parameters of treatment for MDR bacterial infections. The 23.3 percentage-point improvement in clinical cure rate, 30.9 percentage-point improvement in microbiological eradication, 4.4-day reduction in hospital stay, and 71.7% relative reduction in 30-day mortality are statistically and clinically strong.

The ability of these nano-formulations to restore antibiotic susceptibility in resistant pathogens via penetration of biofilms, multi-target antimicrobial activity and improved pharmacokinetics is a fundamentally new and complementary strategy to existing antimicrobial stewardship and resistance containment strategies. The safety profile noted in this trial is encouraging, but continued monitoring for long-term toxicities associated with the nano should be continued.

These results should be confirmed in a multi-center, double-blind phase III study with more patients and in greater geographic distribution. The successful sustainability of clinical implementation for local production of nanobiotics will require concurrent investment in local nanobiotics manufacturing capacity, quality assurance systems, and health-economic evaluation systems. Nano-antibiotics are a promising new battleground in the struggle against antimicrobial resistance, which will shape public health over the coming decades.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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