Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2025; 6(2): 100-105

www.microbiojournal.com
Received: 04-06-2025
Accepted: 03-07-2025

RD Umar
Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi, Nigeria

I Titus
Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi, Nigeria

E Joseph
Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi, Nigeria

UG Mamman
Peake Specialist Hospital, Abuja, Nigeria

Correspondence
RD Umar
Department of Microbiology, Abubakar Tafawa Balewa University, Bauchi, Nigeria

Molecular characterization of multidrug resistance genes and effect of zinc oxide nanoparticles on *Salmonella typhi* serovars from clinical specimens

RD Umar, I Titus, E Joseph and UG Mamman

DOI: https://www.doi.org/10.22271/micro.2025.v6.i2b.242

Abstract
Typhoid fever also known as enteric fever is an acute systemic infection caused by *Salmonella typhi*. Multi-resistance of *Salmonella* spp to antibiotics have increased a great deal, especially in Nigeria due to indiscriminate use. The use Zinc Nanoparticles for antimicrobial activity has shown significant improvements. Multidrug resistant isolates of *Salmonella typhi* were determined. The genomic DNA was extracted and analysed using standard primers. Electrophoresis was performed using 16SrRNA primers. Antimicrobial activity of Zinc Oxide Nanoparticles against *Salmonella typhi* was determined. 42 of *S. typhi* isolates showed Multidrug resistance. Highest resistance was recorded in Methicillin 40(97.6%). Ciprofloxacin showed the highest sensitivity of 35(85.4%). Resistance genes were detected. Zinc Oxide showed antibacterial activity at different concentrations. With the resistance towards traditional antibiotics, fluoroquinolones are the antimicrobial agents of choice in treating MDR *S. typhi*. Continuous surveillance of antibiotics resistance in *Salmonella typhi* is recommended to understand the sources, epidemiology and transmission of MDR.

Keywords: *Salmonella typhi*, nanoparticles, zinc oxide, *Salmonella typhi*, resistance genes, multi-drug resistance

1. Introduction
Salmonella infections have a worldwide distribution and remains a serious problem to public health significance globally [1]. *Salmonella* species are one of the most widespread disease-causing organisms that affect animals and humans worldwide. Typhoid fever is an acute systemic infection caused by *Salmonella typhi*, a gram-negative bacterium [2]. Typhoid fever causes substantial economic loss resulting from mortality, morbidity and poor growth with hazard of transmitting food poisoning with gastroenteritis and enteric fever to humans [3].
Typhoid causes significant health problems in developing countries due to their unsuitable sewage treatments, poor standards of hygiene and unavailability of potable drinking water [4, 5]. It is mostly encountered in tropical and sub-tropical countries including Nigeria where it constitutes significant sources of morbidities and mortalities [6]. Developed countries have brought the incidence of typhoid fever to very low levels due to infrastructure advances.
Fever is the most common cause of consultations in the tropics and sub tropics where most fever are of infectious and unknown origins of which typhoid accounts for a majority. Typhoid fever is among the endemic diseases [7]. Typhoid fever is highly prevalent in Nigeria and other sub-Saharan Africa where the highest population of world's poor live [8]. The disease is an indication of neglect of control of the environment, while it is going extinct in the wider world, the case in Africa remains an alarming one as it is been recorded to constitute a major cause of hospital admissions in Africa [9].
Multidrug resistant *Salmonella typhi* (*Salmonella typhi* that are resistant to 2 or more antibiotics) has caused outbreaks in several countries in the developing world, resulting in increased morbidity and mortality. The disease is especially high in children below 5 years of age and those who are mal-nourished [10]. Typhoid fever is a systemic disease, without therapy, the illness may last for three to four weeks and death rate ranges between 12% and 30%. Although the global burden of typhoid fever has reduced, emergence of multidrug-resistant *Salmonella typhi* (MDRST) is still a threat to public health. Despite the emergence of newer antibacterial drugs, enteric fever has continued to be a major health problem [11].

Examining the antibiotic susceptibility patterns of pathogens is important toward tailoring treatment to the ever-changing resistance patterns and distribution of pathogenic bacteria.

Recent developments in the mapping of *salmonella* genome have provided insights into its pathogenicity, virulence and how antibiotic resistance work in human immunity develop.

There is a great regional variability with regard to antibiotic sensitivity and the presence of MDR strains because misuse of antibiotics is a potent cause of the development of MDR strains [12]. Therefore, it is essential that physicians working in regions where typhoid fever is endemic to ascertain the nature and prevalence of the different strains and base appropriate recommendations for the first and second-line therapy.

Modern drug delivery system is now based on the science and technology of the highly beneficial principle of site-specific or targeted therapy. This has been revolutionized by the development and advancements made in nanotechnology. The use of nanoparticles and zinc specifically in various medical applications has allowed for huge progress and developments in cancer treatment, drug therapy and other applications related to visualization, sensing, and gene delivery [13]. Leveraging on this, there is much to be gained from the use of zinc nanoparticles for treatment, as this form of treatment has many advantages over conventional drug therapy. A significant factor is the ability of nanoparticles to target specific areas, thus avoiding non-specific interactions of the drug with unaffected tissues and thus prevention of undesired side effects [14]. Among the favourable properties of Zinc nanoparticles, the ability to self-assemble, high stability in biological systems, specificity concerning tissue targeting and the ability to encapsulate drugs stand out [15].

Antibiotics are currently being rendered obsolete by the growing incidence of drug resistance among disease causing bacteria. Research must be focused on developing solutions to this problem by creating new drugs and chemically altering existing ones. However, the rate of evolution of drug resistance far outstrips efforts to impede this phenomenon in the laboratory [16]. Nanotechnology offers solution to these limitations in current treatment methods due to the relatively small size of nanoparticles in comparison to biological molecules.

The answer to the problem of antimicrobial resistance may lie in the relatively recent discovery of antimicrobial nanoparticles against which the pathogens may not be able to develop resistance mechanism. The nano-materials themselves would act as precisely engineered platforms from which drugs may be delivered to target physiological sites [17]. Zinc oxide nanoparticles is highly effective on pathogens and has no cytotoxic effects, it has also been incorporated into several biomedical and industrial processes in the food industry and in wastewater treatment [18].

2. Materials and Methods

2.1 Study area

This study was conducted in selected hospitals across Bauchi metropolis. The study was carried out on both Febrile and Diarrhoeic patients of all ages and sex attending these hospitals. The blood and stool specimens were collected from patients diagnosed by physicians with either fever, gastroenteritis or both. Patients' information and Bio data was recorded.

2.2 Collection and processing of specimen

About 436 specimens of blood and stool were collected from three [3] selected hospitals in Bauchi Metropolis to determine the presence of *Salmonella typhi*. Fluid Tetrathionate (8ml) broth in McCartney bottles was used to collect 2ml of venous blood. One (1) gram of stool sample was dispensed in nine (9ml) of Selenite Broth F (PART Part II and I) and used for pre-enrichment. The specimen was incubated was at 37°C for 24 and 48 hours. Turbidity was observed for growth.

One (1) gram of stool specimen was placed in Selenite F Broth and incubated for 24hrs at 37°C then sub cultured onto Salmonella-Shigella Agar, MacConkey agar and Brilliant Green agar, then incubated for 24hrs at 37°C. The representative *Salmonella* colonies were characterized phenotypically using Gram' staining [19].

Two (2) ml of venous blood was drawn aseptically from the patients and placed into 8ml of tetrathionate broth. The culture broths were incubated at 37°C and checked for signs of bacterial growth daily for up to ten days. Bottles with signs of growth were sub cultured onto Salmonella-Shigella Agar, Brilliant Green agar and MacConkey agar.

2.3 Biochemical screening and serotyping

Biochemical characteristics of the isolates were determined using Sugar fermentation tests, Urease test and Catalase test. Colonies considered to be of *Salmonella* spp. were further tested for Somatic (O) and Flagella (H) antigens with polyvalent antisera.

2.4 Antimicrobial susceptibility testing and Multidrug Resistant screening

Antimicrobial susceptibility pattern of the isolates was carried out using the disc diffusion method. Mueller-Hinton agar was used to culture the isolates and test them against the following antibiotics, Cephalothin (CEF, 5mg), Cefuroxime (CXM, 30mg), Amoxicillin (AMC, 30mg), Erythromycin (ERY, 25mg), Augmentin (AUG, 30mg), Ceftriaxone (CFX, 30mg), Cefotaxime (CTX, 30mg), Ciprofloxacin (CIP, 10mg), Amikacin (AMK, 10mg), Neomycin (NEO, 30mg), Ampicillin (AMP, 10mg), Chloramphenicol (CHL, 5mg), Nalidixic acid (NAL, 5mg), Cotrimoxazole (COT, 25mg), Fusidic acid (FA, 5mg), Colistin sulphate (CST, 25mg), Gentamycin (GEN, 10mg), Novobiocin (NOV, 5mg), Oxacillin (OXA, 5mg) Methicillin (MET, 5mg), Tetracycline (TET, 30mg) and Imipenem (IPM, 10mg). Isolates that are resistant to 2 or more antibiotics were screened and classified as Multidrug resistant

2.5 DNA extraction and electrophoresis of extracted *Salmonella typhi* DNA products

The genomic DNA was extracted using Accuprep® extraction kit (Cat. K-3032) ISO 9001 and analysed using standard primers for the PCR analysis. The bacterial cell-1 x 10^9 was collected by centrifuging at 8000 rpm for 5min. the supernatant (media) was discarded by using a pipette. 180 µl of TL Buffer was added to the collected pellets and completely resuspended by vortexing or pipetting. 20 µl of Proteinase K and 10 µl of RNase A were mixed by vortexing and incubated at 60ºC for 1 hour. 200 µl of GB Buffer was added and immediately mixed by vortexing. The agarose gel electrophoresis was performed using 16SrRNA standard primers: 25F:5'-AGAGTTTGATCMTGGCTCAG-

3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3'. The PCR condition for initial denaturation was 10 minutes at 92°C and 30 cycles consist of denature at 92°C for 45s; annealing at 50°C for 15s, extension at 72°C for 2 minutes; and final extension at 72°C for 2 min. The final PCR product was resolved on 1% agarose gel electrophoresis (Lee *et al*., 2009) [10]. The supernatant contains the DNA needed for electrophoresis assay, and was stored at 20°C.The concentration and purity of the extracted DNA was estimated using a Nano drop spectrophotometer and the integrity of the DNA was assessed by 1.2% agarose gel electrophoresis [14].

2.6 Resistant genes profiling

Salmonella typhi isolates screened for MDR phenotype and characteristic were used for resistant genes determination. The resistance genes, Tem-1, Sul-1, Gyr-A, and Cat-1 were investigated. DNA analysis and multiplex PCR was carried out with synthesized primers.

2.7 Antibacterial susceptibility assay of zinc oxide nanoparticles

The ZnO Nanoparticles was purchased and dissolved in sterilized distilled water. 0.2, 0.4, 0.6, 0.8 and 1 mg/ml concentrations of ZnO NPs were vigorously vortexed before performing the experiment. Disc diffusion assay was used to analyse the antimicrobial activity of ZnO Nanoparticles against *Salmonella typhi*.

The diluted culture was swabbed on prepared Muller Hinton agar plates. Sterile filter paper disks of Whatman filter, 5mm in diameter was used for disc diffusion assay. These blank discs were impregnated with (0.2, 0.4, 0.6, 0.8 and 1) mg/ml concentrations of ZnO NPs and allowed to diffuse radially across the disc. Standard discs of Amoxicillin (10mg) and augmentin (5 mg) were used as positive control while a blank disc (impregnated with water) was used as negative control, respectively [17].

3. Results and Discussion

This study evaluated the frequency distribution of *S. typhi* isolates and MDR according to patient's demographic information and bio-data (Table 1), Age group 31-40 and 0-10 had the highest frequencies of occurrence [15] and (09) while age group 51-60 had the least (1). The result indicated that prevalence of typhoid fever in endemic areas is considered high in young adults and school aged children. Older adults are presumably relatively resistant due to frequent boosting of immunity. The study according to gender showed that 164 specimens were collected for males and 272 for females, highest frequency of *S. typhi* was found within the Female gender [22] while Males recorded the lowest [19]. Females are more vulnerable to such diseases due to the physiological status, hormonal imbalance and changes as well as environmental factors associated to these women. Both Out-patients [26] and In-patients [15] were recorded in the present study. It also indicated that outpatients had the most percentage of *Salmonella typhi* isolated than the in patients Table 3. The high rates among the out-patient were attributed to self-medication and indiscriminate use of antibiotics before coming to the hospital thereby engaging in irrational treatment that may not be effective.

Table 1*:* Distribution of *Salmonella typhi* isolates and MDR according to patients' demographic information

Patients' information	No of Samples collected, (N=436)	No (%) of *S. typhi* isolates, (N=41)	No (%) of MDR *S. typhi* isolates, (N =32)
Age (years)			
0-10	129	09	08
11-20	67	03	03
21-30	42	07	05
31-40	83	15	12
41-50	33	04	02
51-60	44	01	01
>70	38	02	01
Sex/Gender			
Female	272	22	18
Male	164	19	14
Group			
Out-patient	298	26	22
In-patient	138	15	10

Enteric pathogens are the principal causes of diarrhoea and fever in both adults and children. This study observed that other organisms isolated from the specimens collected are of the enterobacteriaceae family. The distribution showed *Escherichia coli* with the highest prevalence rate of 26.6%, *Klebsiella* species 14.2%, *Shigella* species 13.3%, *Salmonella* species 9.4%, *Pseudomonas* species 10.8% *and Proteus* species 10.1%. *Enterobacter* species had the least percentage of 13.3% (Table 7).

Table 2: Frequency of occurrence of Enterobacteriaceae isolates from the specimens collected from patients in the study area

Isolates	Number of occurrences (N=436)	Percentage (%)
Escherichia coli	116	26.6
Klebsiella sp.	62	14.2
Shigella sp.	58	13.3
Salmonella typhi.	41	9.4
Pseudomonas sp.	47	10.8
Proteus sp.	44	10.1
Enterobacter sp.	68	13.6

Serovar of *S. typhi* with Multidrug resistance were observed in this study. Multi-Drug Resistant (MDR) pathotypes are now frequently encountered, making treatment difficult and less effective. Resistance traditionally efficacious drugs are now being reported globally for example resistance to ampicillin, chloramphenicol, cotrimoxazole, and ciprofloxacin were observed in this study.

Table 3: Distribution of isolates according to antibiotic susceptibility pattern

Antimicrobial Agent (µg)	Number (%) of isolates (N=41) and susceptibility pattern (%)	
	Sensitive	Resistant
Ampicillin (10)	12 (29.3)	29 (70.7)
Augmentin (30)	15 (36.6)	26 (63.4)
Amikacin (30)	12 (29.3)	29 (70.7)
Ceftriaxone (30)	27 (65.9)	14 (34.1)
Cefuroxime (30)	17 (41.5)	24(58.5)
Cefotaxime (30)	11 (26.8)	30 (73.2)
Cephalothin (5)	18 (43.9)	23 (56.1)
Chloramphenicol (5)	20 (48.8)	21 (51.2)
Ciprofloxacin (5)	35 (85.4)	6(14.6)
Cotrimoxazole (25)	22 (53.7)	19 (46.3)
Colistin Sulphate (25)	20 (48.8)	21 (51.2)
Erythromycin (25)	10 (24.4)	31(75.6)
Fusidic acid (5)	13 (31.7)	28(68.3)
Gentamycin (10)	13 (31.7)	28(68.3)
Imipenem (10)	06(14.6)	35 (85.4)
Novobiocin (5)	09(22.0)	32 (78.0)
Oxacillin (5)	05(12.2)	36 (87.8)
Methicillin (5)	01 (2.4)	40(97.6)
Tetracycline (30)	11(26.8)	30 (73.2)

This study revealed that 41 of *S. typhi* isolates were resistant to two (2) or more antimicrobial agents (Multidrug resistance). Highest resistance was observed in Methicillin 40(97.6%), while the isolates were sensitive to Ciprofloxacin 35(85.4%). Highest susceptibility of *Salmonella typhi* was seen against Ciprofloxacin. However, in Lagos, Nigeria, [22] reported 18% reduced susceptibility of *Salmonella* spp to Ofloxacin and Ciprofloxacin. The development of resistance almost certainly comes from the use of cheaper generic drugs in the treatment of bacterial infections. The relatively low cost of generic medicine is partly the major reason for antibiotics abuse [24].

Six (6) isolates were selected for molecular studies to determine the presence of resistant genes Sul, Tem, Cat and Gyr-A. The multiplex PCR was performed for Sul and Tem while single PCR was performed for Cat and Gyr-A resistant genes respectively. Results obtained showed the presence of all resistant genes in this study. This is indicative of the high prevalence of resistance present among populations and communities partly due to antibiotics misuse and abuse.

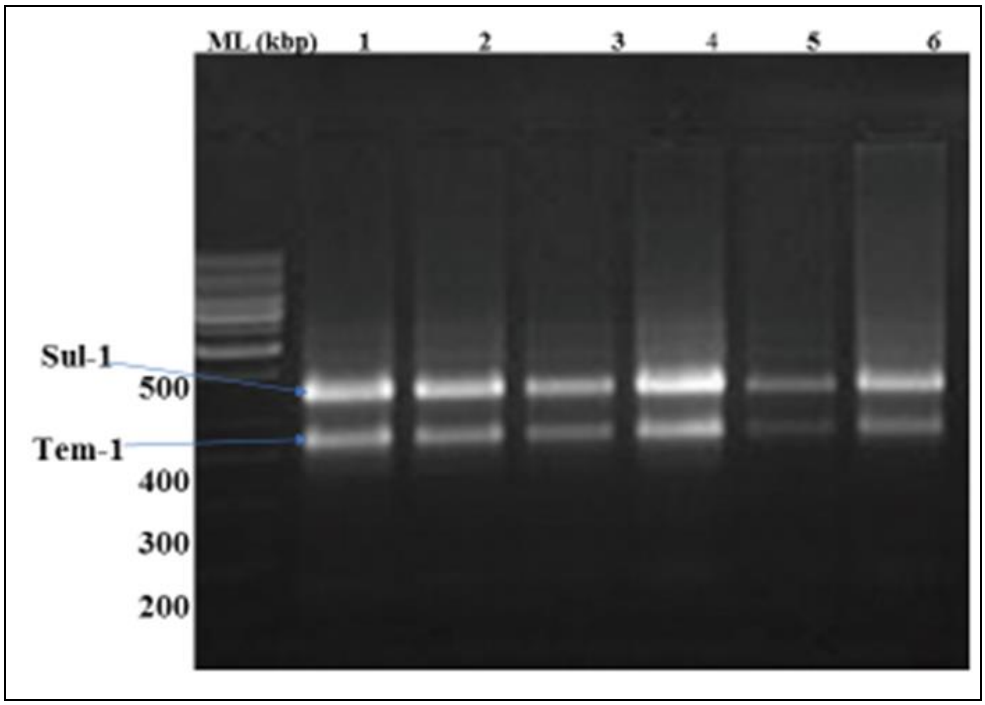

Fig 1: Gel electrophoresis profile for Sul-1 and Tem-1 genes in MDR *Salmonella typhi*

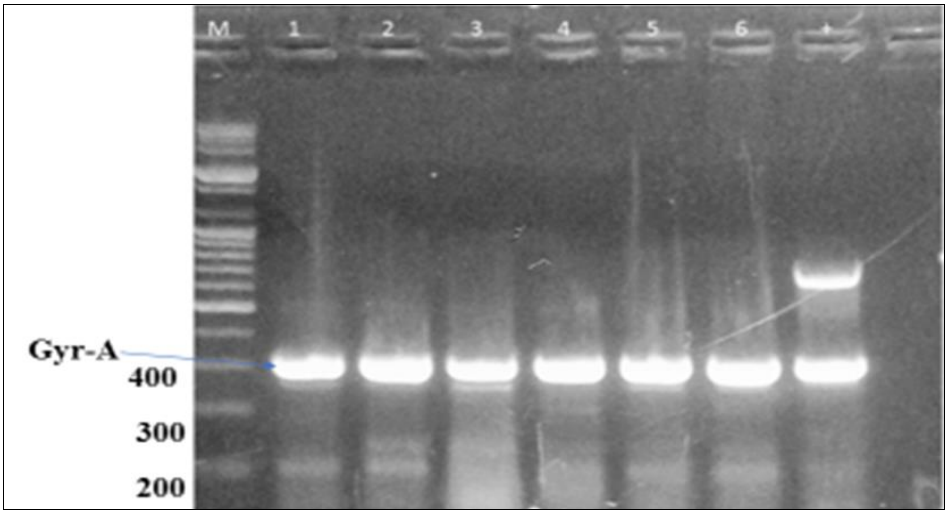

Fig 2: Gel electrophoresis profile for Gyr-A gene in MDR *Salmonella typhi*

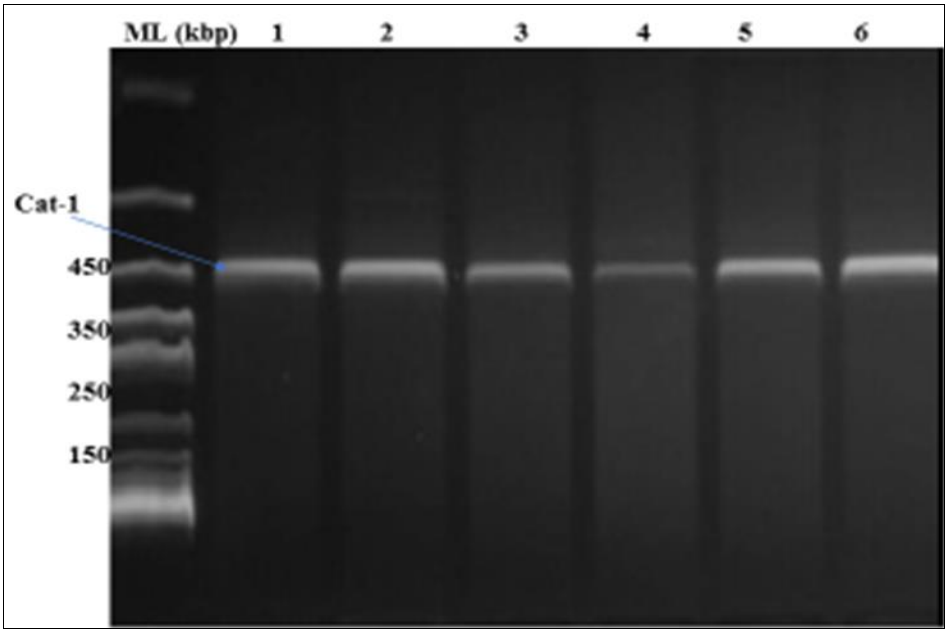

Fig 3: Gel electrophoresis profile of Cat-1 gene in MDR *Salmonella typhi*

Table 3: Zones of Inhibition of zinc oxide nanoparticles against *Salmonella typhi* isolates

Concentration(mg/ml) and Zones of Inhibition (mm)					
Isolates	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
ST 01	9.0	11.0	12.0	15.2	22.0
ST 02	8.0	10.0	15.0	20.0	23.2
ST 03	10.0	11.3	17.0	21.5	23.0
ST 04	7.0	9.0	13.0	15.0	24.0
ST 05	11.0	15.0	16.0	19.0	21.2
ST 06	10.0	12.5	15.0	16.0	22.3

3.1 Antibacterial activity of Zinc Oxide nanoparticles on *Salmonella typhi*

Antibacterial activity of ZnO nanoparticles carried out on selected isolates to determine the efficacy of Zinc oxides on *Salmonella typhi* showed that ZnO NPs were able to inhibit the bacterial growth at all given concentrations with the maximum zone of inhibition at 1 mg/ml concentration i.e. 23.2 mm in *ST O2 isolate* and 22.3 mm in ST 06 isolate. The lowest inhibitory zones formed at 0.2 mg/ml concentration in ST 04 and ST 02 isolates were measured as 7 mm and 8 mm respectively, as shown in Table 3. The antibacterial activity of ZnO NPs in this study indicated increases as the concentration of NPs increases and maximum inhibitory zone is obtained at 1 mg/ml concentration of ZnO NPs for all strains of *Salmonella typhi* used for this study.

4. Conclusion

Salmonella typhi was isolated in this study and found to be more prevalent in middle age group, female outpatients. Multidrug resistance strains were isolated from our study. Multidrug resistant serovars of *Salmonella* has added to the urgent need for the development of more effective control measures. Tem-1, Sul-1, Gyr-A, and Cat-1 resistant genes

were detected in all the MDR strains in this study. Zinc oxide nanoparticles inhibited the growth of *Salmonella typhi* at all concentrations used and creating the maximum zone of inhibition at 1mg/ml concentration (23.2). Findings of this study revealed that antibacterial activity of Zinc oxide nanoparticles increases as the concentration of nanoparticle increases.

5. Consent and ethical approval
Ethical approval was sought obtained from the Government of Bauchi State, Ministry of Health research and ethics committee, with written informed consent also sought from all patients prior to specimen and data collection.

6. Acknowledgements
The researchers wish to thank Tetfund Institutions Based Research (IBR) Ref. No. TETF/DR&D/CE/UNI/BAUCHI /IBR/2022/VOL.1for the fund to carry out the research and Directorate of Research and Innovations of Abubakar Tafawa Balewa University Bauchi for the approval and necessary assistance and guidance.

Conflict of Interest
Not available

Financial Support
Not available

7. References
1. World Health Organization. Economic cost of typhoid fever. Salmonella control program report [Internet]. 2021 [cited 2021 May 1]. Available from: https://who.typhoid-fever
2. Sehra D, Sehra S, Relia P, Sehra ST. An altered drug resistance pattern in *Salmonella typhi*. Am J Infect Dis Microbiol. 2013;1(5):84-85.
3. Brown AN, Smith K, Samuels TA, Lu J, Obare SO, Scott ME. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and methicillin resistant Staphylococcus aureus. Appl Environ Microbiol. 2012;78:2768-2774.
4. Michael CA, Howes DD, Labbate M. The antimicrobial resistance crisis: causes, consequences, and management. Front Public Health. 2014;2:145.
5. Gindy ME, Prud'homme RK. Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. Expert Opin Drug Deliv. 2009;6(8):865-78. DOI: 10.1517/17425240902932908.
6. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009;48:1-12.
7. Cheesbrough M. Salmonella species. In: District Laboratory Practice in Tropical Countries. Cambridge: Cambridge University Press, 2006.
8. Collee JG, Marmion BP, Fraser AG, Simmons AM. Mackie and McCartney Practical Medical Microbiology. 14th Ed. Edinburgh: Churchill Livingstone Inc., 1996, p. 978.
9. Perilla M, Ajello G, Bopp C, Elliott J, Facklam R. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Geneva: WHO, 2003.
10. Lee YH, Chi MH, Park SY. A quick and safe method for fungal DNA extraction. Plant Pathol J. 2009;25(1):108-111.
11. Asma H, Ali A, Sarwar Y, Mohsin M, Bashir S, Tariq A. Multiplex PCR for differential diagnosis of emerging typhoidal pathogens directly from blood samples. Epidemiol Infect. 2009;137(1):102-107.
12. Thangam R, Sathuvan M, Poongodi A, Suresh V, Pazhanichamy K, Sivasubramanian S, et al. Activation of intrinsic apoptotic signaling pathway in cancer cells by *Cymbopogon citratus* polysaccharide fractions. Carbohydr Polym. 2014;107:138-150.
13. Enemchukwu BN, Ibe CC, Udedi SC, Iroha A, Ubaoji KI, Ogundapo SS. Liver function assessment in malaria, typhoid and malaria-typhoid co-infection in Aba, Abia State, Nigeria. Pak J Biol Sci. 2014;17(6):860-3. DOI: 10.3923/pjbs.2014.860.863.
14. Alanis AD, Calzada F, Cervantes JA, Torres J, Ceballos GM. Antimicrobial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol. 2005;100:153-157.
15. Zaki SA, Karande S. Multidrug-resistant typhoid fever: A review. J Infect Dev Ctries. 2011;5(5):324-37. DOI: 10.3855/jidc.1405.
16. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi, United Kingdom. Emerg Infect Dis. 2001;7(3):448.
17. Akinyemi KO, Bamiro BS, Coker AO. Salmonellosis in Lagos, Nigeria: Incidence of Plasmodium falciparum-associated co-infection, patterns of antimicrobial resistance, and emergence of reduced susceptibility to fluoroquinolones. J Health Popul Nutr. 2007;25(3):351-358.
18. Uzzau S, Figueroa-Bossi N, Rubino S, Bossi L. Epitope tagging of chromosomal genes in Salmonella. Proc Natl Acad Sci USA. 2001;98(26):15264-15269.
19. Siddiqi KS, Husen A, Rao RAK. A review on biosynthesis of silver nanoparticles and their biocidal properties. J Nanobiotechnol. 2018;16:1-10.

How to Cite This Article
Uma RDR, Titus I, Joseph E, Mamman UG. Molecular characterization of multidrug resistance genes and effect of zinc oxide nanoparticles on *Salmonella typhi* serovars from clinical specimens. Journal of Advances in Microbiology Research. 2025;6(2):100-105.