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Prevalence and distribution of the World Health Organization-Bacterial Priority Pathogens in selected hospitals in Katsina State, Nigeria

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Abstract:

Background: Infections caused by antibiotic-resistant bacteria have become a major global public health challenge, as they can reduce the effectiveness of modern medical treatments and clinical care. The most important of these pathogens are those listed in the World Health Organization (WHO) Bacterial Priority Pathogens List (BPPL) of 2024. This study determined the prevalence, distribution, and antimicrobial resistance patterns of the WHO bacterial priority pathogens in three selected hospital settings in Katsina State, Nigeria.

Methodology: We conducted a descriptive cross-sectional targeted survey of the WHO bacterial priority pathogens in 230 clinical samples from patients with suspected bacterial infections. Bacterial isolation and identification were performed using standard microbiological procedures. The resistance patterns were determined after performing antimicrobial susceptibility testing on the isolates in accordance with the 2024 CLSI guidelines. Further characterization of the pathogens was done using phenotypic resistance detection methods, including the double disk synergy test (DDST), Carba NP, and MRSA detection tests.

Results: Of the 230 samples, 113 samples (49.1%) yielded bacterial growth, with Gram-negative bacteria accounting for 58.4% of the isolates. The most frequently isolated bacterium was *Klebsiella aerogenes* (22.1%), followed by *Escherichia coli* (20.4%). The highest frequency of isolates (29.2%) was obtained from urine samples. The isolated bacteria showed high resistance to most antibiotics tested, with resistance rates to imipenem and cephalexin ranging from 78.3% to 100%, however gentamicin against Gram-negative and vancomycin against Gram-positive isolates were relatively effective. The overall prevalence of multidrug resistance (MDR) was 91.8% and the multiple antibiotic resistance (MAR) index value of all the isolates was greater than 0.2, which indicated exposure to high-risk sources of antimicrobial pressure. The WHO priority bacterial pathogens accounted for 77.0% of the total isolates. The most prevalent were third-generation cephalosporin-resistant Enterobacteriaceae (39.8%).

Conclusion: The WHO bacterial priority pathogens with high antimicrobial resistance are present in hospitals in Katsina State, Nigeria. These findings highlight the necessity of enhancing laboratory diagnostic capacity, routine culture and sensitivity testing before antibiotic administration, implementing effective antimicrobial stewardship programs, establishing regulations limiting over-the-counter antibiotic sales, and strengthening infection control and prevention practices within healthcare facilities in the State.

Keywords: Antimicrobial resistance, WHO, Bacterial priority, Multidrug-resistant bacteria, Carbapenem-resistance

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Prévalence et répartition des agents pathogènes bactériens prioritaires de l'Organisation Mondiale de la Santé dans certains hôpitaux de l'État de Katsina, au Nigéria

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Résumé:

Contexte: Les infections causées par des bactéries résistantes aux antibiotiques constituent un enjeu majeur de santé publique mondiale, car elles peuvent réduire l'efficacité des traitements médicaux modernes et des soins cliniques. Les pathogènes les plus importants sont ceux répertoriés dans la Liste des pathogènes bactériens prioritaires (LPBP) de l'Organisation mondiale de la Santé (OMS) de 2024. Cette étude a déterminé la prévalence, la distribution et les profils de résistance aux antimicrobiens des pathogènes bactériens prioritaires de l'OMS dans trois établissements hospitaliers sélectionnés de l'État de Katsina, au Nigéria.

Méthodologie: Nous avons mené une étude descriptive transversale ciblée sur les pathogènes bactériens prioritaires de l'OMS dans 230 échantillons cliniques provenant de patients présentant une suspicion d'infection bactérienne. L'isolement et l'identification des bactéries ont été réalisés selon les procédures microbiologiques standard. Les profils de résistance ont été déterminés après réalisation de tests de sensibilité aux antimicrobiens sur les isolats, conformément aux directives CLSI de 2024. Une caractérisation plus poussée des pathogènes a été effectuée à l'aide de méthodes de détection phénotypique de la résistance, notamment le test de synergie à double disque (TSDD), le test Carba NP et le test de détection du SARM.

Résultats: Sur les 230 échantillons, 113 (49,1%) ont présenté une croissance bactérienne, les bactéries Gram négatif représentant 58,4% des isolats. La bactérie la plus fréquemment isolée est *Klebsiella aerogenes* (22,1%), suivie d'*Escherichia coli* (20,4%). La plus grande proportion d'isolats (29,2%) a été obtenue à partir d'échantillons d'urine. Les bactéries isolées présentaient une forte résistance à la plupart des antibiotiques testés, avec des taux de résistance à l'imipénem et à la céphalexine allant de 78,3% à 100%. En revanche, la gentamicine contre les isolats Gram négatif et la vancomycine contre les isolats Gram positif se sont révélées relativement efficaces. La prévalence globale de la multirésistance (MDR) était de 91,8% et l'indice de résistance à de multiples antibiotiques (MAR) de tous les isolats était supérieur à 0,2, ce qui indique une exposition à des sources de pression antimicrobienne à haut risque. Les pathogènes bactériens prioritaires de l'OMS représentaient 77,0% du total des isolats. Les plus prévalents étaient les entérobactéries résistantes aux céphalosporines de troisième génération (39,8%).

Conclusion: Les pathogènes bactériens prioritaires de l'OMS présentant une forte résistance aux antimicrobiens sont présents dans les hôpitaux de l'État de Katsina, au Nigéria. Ces résultats soulignent la nécessité de renforcer les capacités de diagnostic en laboratoire, de réaliser systématiquement des cultures et des antibiogrammes avant l'administration d'antibiotiques, de mettre en œuvre des programmes efficaces de bon usage des antibiotiques, d'établir une réglementation limitant la vente d'antibiotiques sans ordonnance et de renforcer les pratiques de prévention et de contrôle des infections au sein des établissements de santé de l'État.

Mots-clés: Résistance aux antimicrobiens, OMS, bactéries prioritaires, bactéries multirésistantes, résistance aux carbapénèmes

Introduction:

The rising prevalence of bacterial infections that no longer respond to commonly used antibiotics has become a major public health issue worldwide. Antimicrobial resistance (AMR) threatens the efficacy of current treatment options and complicates the management of infectious diseases in both community and hospital settings (1). The spread of resistant microorganisms has not only resulted in longer hospital stays but also in increased mortality and treatment costs.

Poor hygiene practices, lack of awareness of infection prevention and control, improper use of antibiotics, and insufficient surveillance systems to monitor antimicrobial resistance are among the contributing factors to the high burden of antibiotic-resistant infections in

Nigeria (2). Antibiotics are often administered in most healthcare institutions without laboratory confirmation of susceptibility against the causative pathogens and this situation only increases the prevalence of resistant strains (3).

In response to the growing global challenge of AMR, the WHO released an updated Bacterial Priority Pathogens List (BPPL) in May 2024. This list highlights bacterial species that pose the most serious risk to human health and is intended to support research, strengthen surveillance, and the development of new antimicrobial agents. The updated list includes 24 bacterial pathogens across 15 families and classifies them into three priority levels; critical, high, and medium. The classification of these pathogens is based on several criteria, which include severity of infections caused, mortality rate, disease burden, transmissibility, resistance trends, availability of effective treatment

options, and preventability in healthcare and community settings. Other considerations include the availability of new antibiotics currently under development and potential public health impact of the pathogens (2,4,5).

Pathogens classified in the critical priority group are carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem-resistant Enterobacterales (CRE), third-generation cephalosporin-resistant Enterobacterales, and rifampicin-resistant *Mycobacterium tuberculosis*. The high-priority group comprises fluoroquinolone-resistant *Salmonella* spp and *Shigella* spp, third-generation cephalosporin-resistant *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*, and vancomycin-resistant *Enterococcus faecalis*. These pathogens are usually associated with healthcare-associated infections and are very difficult to treat. The medium-priority group includes macrolide-resistant Group A *Streptococcus*, macrolide-resistant *Streptococcus pneumoniae*, penicillin-resistant Group B *Streptococcus*, and ampicillin-resistant *Haemophilus influenzae* (2).

Globally, it has been estimated that approximately 700,000 deaths occur annually as a result of drug-resistant infections, largely associated with the WHO-BPPL, and if effective interventions are not implemented, this number could rise to about 10 million deaths each year by 2050, with nearly 4.1 million deaths expected to occur in Africa alone. In addition to its effects on health, AMR will result in significant global economic losses (6,7).

In Nigeria, particularly in Katsina State, there is limited empirical data or research evidence on the epidemiology of the WHO bacterial priority pathogens. Various studies have indicated that their epidemiology is poorly documented, particularly in hospital settings (8). Such limited empirical data prevent the healthcare provider from taking effective steps to control infections and restrict the ability of government to develop specific interventions in the sphere of public health. WHO has also highlighted that region-specific data on the epidemiology of these pathogens are important for understanding the burden of disease, planning effective infection prevention and control activities, guiding antimicrobial stewardship, and supporting the formulation of disease-specific treatment guidelines (7).

Like many other States in Nigeria, Katsina faces growing issues with AMR and hospital-acquired infections. The emergence and spread of multidrug-resistant (MDR) bacterial pathogens have led to increased morbidity, mortality, and health care expenditures. Poor anti-

biotic use, poor infection control practices, and limited diagnostic capacity are among the factors that still contribute to the spread of resistant bacteria in healthcare facilities (3,9,10).

The inadequate comprehensive data on these pathogens complicate the development of effective interventions and policies to limit their effects and transmission. It is therefore necessary to provide credible information on their prevalence, distribution, and resistance patterns to enhance infection control practices and standard patient management. This study, by providing information on the prevalence and distribution of the WHO bacterial priority pathogens in selected hospitals in Katsina State, contributes to the initiative in understanding the local burden of AMR. The results will be useful for decision-making, antimicrobial stewardship programs, and infection prevention and control practices in healthcare facilities in the State. Finally, this research will contribute to national and global strategies to reduce the impact of drug-resistant infections.

Materials and method:

Study design and setting:

The study was a hospital-based descriptive cross-sectional survey in three selected hospitals located in Katsina State, Nigeria. The three hospitals were Federal Teaching Hospital Katsina, General Hospital Kurfi, and General Hospital Dutsinma.

Study participants and selection:

Patient recruitment and sample collection were carried out within different clinical units of these hospitals, including accident and emergency units, medical and surgical wards, pediatric wards, laboratory units, and other relevant hospital departments. Participants included in the study consisted of patients of all age groups with suspected bacterial infections, regardless of gender, who were not on any antibiotic therapy or had not received antibiotics in the preceding two weeks before the study. Participants who were on antibiotic therapy or had received antibiotics in the 2 weeks preceding the study, and those with other medical conditions other than bacterial infections were excluded.

Ethical considerations:

The study was conducted after obtaining ethical approval from the ethical committee chairman at each of the selected State hospitals and from the Ministry of Health in Katsina State,

with ethical approval number MOH/ADM/SUB/1152/1/1064. Ethical approval was also obtained from the Federal Teaching Hospital, Katsina, with the assigned number FTHKHRECDEG24/06/22C/248. Strict confidentiality was maintained throughout the study.

Sample size determination:

The sample size for the study was determined using the standard sample size estimation formula for cross-sectional study design. The calculation was based on the previously reported prevalence rate of bacterial infections of 28.3% in a study done by Mouanga-Ndzime et al., (11) and the calculated sample size was increased by 10% to account for attrition due to contaminated specimens, non-response, or missing data, resulting in an adjusted sample size of 230.

Sample collection and processing:

The clinical specimens collected from enrolled participants included blood, urine, stool, vaginal and wound swabs, using standard aseptic techniques. The specimens were appropriately labeled, and transported to the laboratory in accordance with stipulated guidelines.

Isolation and identification of bacterial pathogens:

To isolate the bacteria, clinical samples were inoculated on the appropriate culture media that included Blood agar, MacConkey agar, Cetrimide agar, and Nutrient agar using sterile procedures. The inoculated plates were incubated aerobically at 37°C for 24 hours. The morphological characteristics of bacterial colonies were observed for colony size, shape, margin, elevation, color, and haemolytic patterns (12).

Gram staining was performed to identify Gram-positive and Gram-negative bacteria. Additional identification was performed using different biochemical tests as appropriate, including catalase, coagulase, motility, indole production, urease activity, citrate utilization, oxidase test, methyl red test, Vogue-Proskauer test, bile solubility, optochin and bacitracin susceptibility.

The procedures were performed in accordance with the microbiological guidelines outlined by Cheesbrough (12). Distinct colonies were sub-cultured to isolate pure cultures, which were stored for later use in bacterial identification and antimicrobial susceptibility testing.

Antimicrobial susceptibility testing of isolates:

The Kirby-Bauer disk diffusion antimicrobial susceptibility test (AST) was performed

on Mueller-Hinton (MH) agar plate. Pure bacterial colonies were resuspended in 5 ml of sterile saline, and the suspension was adjusted to a turbidity of 0.5 McFarland standard. A sterilized swab was then used to spread the standardized culture over the surface of MH agar plates to ensure uniform growth of bacterial colonies. The isolated bacteria were inoculated onto plates containing standard antibiotic discs including cephalosporin (ceftriaxone 30µg, cephalexin 30 µg), carbapenem (meropenem 10µg, imipenem 10µg), fluoroquinolone (ciprofloxacin 5µg), aminoglycoside (gentamicin 15µg), and cotrimoxazole (10µg), with penicillin (amoxicillin-clavulanic acid (20/10µg), glycopeptide (vancomycin 30µg) and macrolide (azithromycin 15µg) for Gram-positive isolates only.

The plates were incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition around each antibiotic disc were measured in millimeters, and the results interpreted as susceptible, intermediate, or resistant based on the Clinical and Laboratory Standards Institute (CLSI) guideline (13).

Determination of multi-drug resistance (MDR) and multiple antibiotic resistance index (MARI):

Multi-drug resistance (MDR) among the bacterial isolates was determined using the criteria set by the Centers for Disease Control and Prevention, which is resistance of bacterial isolates to at least one antibiotic in three or more antibiotic classes (8). In addition, the MAR index was also calculated for each isolate using the formula; MAR index = number of antibiotics to which the isolate is resistant, divided by the total number of antibiotics tested. MAR index values greater than 0.2 were deemed to represent isolates with a background of high antibiotic exposure and high AMR selection pressure (14).

Double-disc synergy test for detection of ESBL production:

The double-disc synergy test (DDST), as previously described by Jarlier et al., (15) and in accordance with the recommendations of CLSI was used to detect ESBL production among the Gram-negative isolates. Cefotaxime (30µg), ceftriaxone (30µg), and ceftazidime (30µg) discs were placed 30 mm apart from an amoxicillin-clavulanate (30µg) disc, and Gram-negative bacterial suspension standardized to a 0.5 McFarland turbidity was inoculated onto MH agar plate. The zone expansion or keyhole effect between the cephalosporin and amoxicillin-clavulanate discs observed 24 hours after incubation

tion at 37°C was used to confirm the production of ESBL (15).

Carba NP test for detection of carbapenemase production:

A rapid colorimetric assay, known as the Carba NP Test, was used to detect carbapenemase production among the Gram-negative isolates. In this method, intracellular enzymes were released after bacterial cells were lysed, and the lysate was then mixed with a solution containing imipenem and the pH indicator phenol red. A color change from red to yellow indicated antibiotic hydrolysis and confirmed carbapenemase production (16).

Detection of fluoroquinolone resistance in *Salmonella* isolates:

Resistance to fluoroquinolones in the *Salmonella* isolates was detected using the disc diffusion method. The suspension was incubated for 18-24 hours at 35°C, and a smaller inhibition zone around the ciprofloxacin disc meant reduced susceptibility or resistance (13, 17).

Detection of methicillin resistance in *Staphylococcus aureus* isolates:

Methicillin resistance in *S. aureus* isolates was detected by uniformly inoculating MH agar plate with a standardized suspension (0.5 McFarland) of overnight culture of *S. aureus* isolates and overlying the surface with a 30 µg cefoxitin disc. The plate was then incubated for 18-24 hours at 35°C, and the diameter of the inhibitory zone was measured. Methicillin-resistant *S. aureus* (MRSA) was identified when the inhibition zone was 21 mm or less, while a zone of 22 mm or more indicated a methicillin-susceptible strain (13).

Detection of vancomycin resistance in *Enterococcus faecalis* isolates:

Vancomycin resistance in *Enterococcus faecalis* was determined by standard AST in accordance with the CLSI guidelines. A bacterial suspension that has been standardized to 0.5 McFarland turbidity was spread out uniformly on the Mueller-Hinton agar plates. On the inoculated agar surface, a vancomycin disc (30 µg) was placed thereafter. An incubation period of 18-24 hours at 37°C was observed on the plates, and the diameter of the inhibition zone surrounding the vancomycin disc was measured and interpreted according to CLSI criteria. Strains that had

lower susceptibility or resistance to vancomycin were considered vancomycin-resistant *Enterococcus* (VRE) (13,18).

Results:

Distribution of bacterial isolates in clinical specimens:

Of the 230 clinical samples collected from patients in the three selected hospitals, 113 samples (49.1%) grew bacteria. Of these, Gram-negative bacteria accounted for 66 isolates (58.4%) and Gram-positive bacteria for 47 isolates (41.6%) (Fig 1).

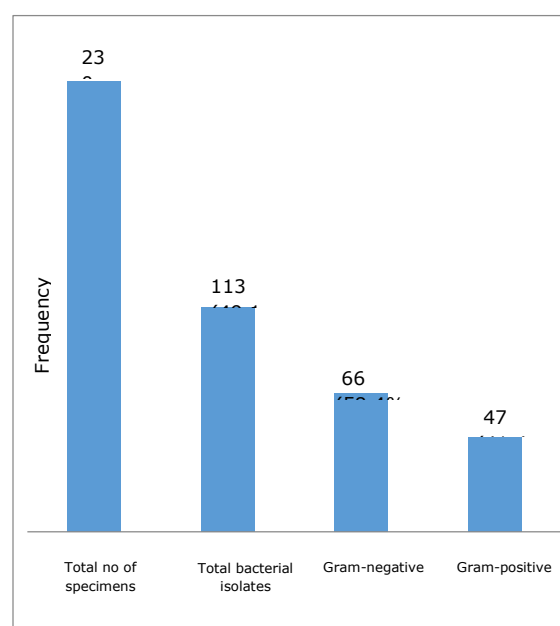


Fig 1: Distribution of bacterial isolates from clinical specimens in selected hospitals in Katsina State, Nigeria

Frequency of bacterial species from clinical specimens of participants:

Of the total of 113 bacterial isolates, the most frequent is *Klebsiella (Enterobacter) aerogenes* with 25 isolates (22.1%), followed by *Escherichia coli*, which accounted for 23 isolates (20.4%). *Proteus vulgaris* was also frequently isolated (10.6%, n=12) and was among the major pathogens identified during the study. Conversely, only one isolate (0.9%) of *A. baumannii* was identified (Table 1).

Table 1: Frequency of bacterial species from selected hospitals in Katsina State, Nigeria

Bacterial pathogen	Number (%)
<i>Klebsiella (Enterobacter) aerogenes</i>	25 (22.1)
<i>Escherichia coli</i>	23 (20.4)
<i>Streptococcus</i> species	15 (13.3)
<i>Klebsiella pneumoniae</i>	13 (11.5)
<i>Proteus vulgaris</i>	12 (10.6)
<i>Enterococcus faecalis</i>	11 (9.7)
<i>Staphylococcus aureus</i>	9 (7.9)
<i>Salmonella</i> species	2 (1.7)
<i>Pseudomonas aeruginosa</i>	2 (1.7)
<i>Acinetobacter baumannii</i>	1 (0.9)
Total	113 (100.0)

Distribution of bacterial isolates according to specimen types:

The distribution of bacterial species also varied according to specimen type. In blood samples, *K. pneumoniae* was the most common pathogen detected accounting for 6 isolates (5.3%), and *A. baumannii* appeared less frequently. In urine samples, *E. coli* was the most common organism with 13 isolates (11.6%) followed by *Streptococcus* species with 2 isolates (1.8%). In stool samples, *E. aerogenes* had the highest number of isolates (n=17, 15.0%), whereas *E. faecalis* had the fewest (n=3, 2.7%). The most common isolates in wound swab were *Streptococcus* species, with 8 isolates (7.1%), followed by *E. aerogenes* with the least number of 1 isolate (0.9%). Only *Streptococcus* species

were recovered from vaginal swab samples as shown in Table 2.

Antibiotic resistance profile of bacterial isolates:

The antibiotic resistance patterns of the bacterial isolates are summarized in Table 3. The analysis showed that there was a high resistance of the isolates to various antibiotics used. It is worth noting that very high rates of resistance to imipenem and cephalixin of between 88% and 100% against the bacterial species isolated was recorded. *Salmonella* species as well as *Pseudomonas aeruginosa* were totally resistant to at least seven out of the ten evaluated antibiotics. Similarly, *S. aureus* was 100% resistant to co-trimoxazole, meropenem, and imipenem.

Gentamicin and vancomycin, however, were highly effective against most of the Gram-negative and Gram-positive bacterial isolates respectively. There was no resistance to gentamicin among *K. aerogenes* while there was also no resistance to vancomycin by *Streptococcus* species, *E. faecalis* and only 14.3% of *S. aureus* were resistant to it. Comparatively low resistance was also observed for most of the isolates to azithromycin except *E. faecalis* (36.4%), *Salmonella* spp (40.0%), *Proteus vulgaris* (66.7%) and *P. aeruginosa* (100.0%).

Table 2: Frequency distribution of bacterial species by specimen types in selected hospitals in Katsina State, Nigeria

Clinical specimens	Bacterial isolates	Number positive (%)
Blood	<i>Escherichia coli</i>	5 (4.4)
	<i>Streptococcus</i> spp	3 (2.7)
	<i>Klebsiella pneumoniae</i>	6 (5.3)
	<i>Proteus vulgaris</i>	2 (1.8)
	<i>Enterococcus faecalis</i>	4 (3.5)
	<i>Staphylococcus aureus</i>	4 (3.5)
	<i>Salmonella</i> spp	2 (1.8)
	<i>Acinetobacter baumannii</i>	1 (0.9)
Urine	<i>Enterobacter aerogenes</i>	7 (6.2)
	<i>Escherichia coli</i>	13 (11.5)
	<i>Streptococcus</i> spp	2 (1.8)
	<i>Klebsiella pneumoniae</i>	3 (2.7)
	<i>Proteus vulgaris</i>	4 (3.5)
	<i>Enterococcus faecalis</i>	4 (3.5)
Stool	<i>Enterobacter aerogenes</i>	17 (15.0)
	<i>Escherichia coli</i>	5 (4.4)
	<i>Klebsiella pneumoniae</i>	4 (3.5)
	<i>Enterococcus faecalis</i>	3 (2.7)
Wound swab	<i>Enterobacter aerogenes</i>	1 (0.9)
	<i>Streptococcus</i> spp	8 (7.1)
	<i>Proteus vulgaris</i>	6 (5.3)
	<i>Staphylococcus aureus</i>	5 (4.4)
	<i>Pseudomonas aeruginosa</i>	2 (1.8)
Vaginal swab	<i>Streptococcus</i> spp	2 (1.8)
Total		113 (100.0)

Table 3: Antibiotic resistance pattern of bacterial pathogens from clinical samples in three selected hospitals in Katsina State, Nigeria

Pathogen/Antibiotics	Number of resistant isolates (%)									
	AMX	AZM	CFM	LEX	CIP	COT	GEN	IMP	MEM	VAN
<i>Escherichia coli</i> (n=23)	5 (21.7)	1 (4.3)	9 (39.1)	20 (86.9)	10 (43.5)	18 (78.3)	1 (4.3)	22 (95.6)	18 (78.3)	NA
<i>Enterobacter aerogenes</i> (n=25)	8 (32.0)	1 (4.0)	12 (48.0)	22 (88.0)	3 (12.0)	20 (80.0)	0	25 (100)	21 (84.0)	NA
<i>Streptococcus</i> spp (n=15)	3 (25.0)	2 (15.4)	6 (43.8)	13 (87.5)	1 (12.5)	10 (68.8)	1 (6.3)	14 (93.8)	7 (50.0)	0
<i>Klebsiella pneumoniae</i> (n=13)	3 (23.1)	2 (8.3)	7 (58.3)	12 (92.3)	2 (15.4)	8 (61.5)	5 (38.5)	13 (100)	11 (84.6)	NA
<i>Enterococcus faecalis</i> (n=11)	4 (36.4)	4 (36.4)	6 (54.5)	11 (100)	3 (27.3)	10 (90.9)	5 (45.5)	9 (81.8)	9 (81.8)	0
<i>Proteus vulgaris</i> (n=12)	8 (66.7)	8 (66.7)	10 (83.3)	11 (91.7)	6 (50.0)	10 (83.3)	6 (50.0)	12 (100)	11 (91.7)	NA
<i>Staphylococcus aureus</i> (n=9)	8 (85.7)	1 (14.3)	8 (85.7)	8 (85.7)	8 (85.7)	9 (100)	0	9 (100)	9 (100)	1 (14.3)
<i>Salmonella</i> spp (n=2)	2 (100)	1 (50.0)	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	NA
<i>Pseudomonas aeruginosa</i> (n=2)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0	2 (100)	2 (100)	NA
<i>Acinetobacter baumannii</i> (n=1)	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	NA

AMX: Amoxicillin, AZM: Azithromycin, CFM: Cefixime, LEX: Cephalixin, CIP: Ciprofloxacin, COT: Co-trimoxazole, GEN: Gentamicin, IMP: Imipenem, MEM: Meropenem, VAN: Vancomycin. NA: Not applicable

Antibiotic resistance phenotypes of the bacterial isolates:

The resistance phenotypes of the bacterial isolates obtained from clinical samples are presented in Table 4. *Salmonella* species, *P. aeruginosa* and *S. aureus* were the isolates with highest resistance phenotypes (resistant to antibiotics in 6 classes), while *Streptococcus* spp on

the other hand, had the least resistance phenotypes (resistant to two antibiotic classes). Generally, the results showed that approximately 90% of Gram-positive bacterial isolates were susceptible to vancomycin and Gram-negative bacteria to gentamicin, indicating that these antibiotics could still be viable treatment options for bacterial infections in the study area.

Table 4: Antibiotic resistant phenotypes of bacterial isolates from clinical samples in three selected hospitals in Katsina State, Nigeria

Bacterial isolate	Resistant phenotype	No of antibiotic class		Number of isolates (%)	Effective antibiotics (based on susceptibility)
<i>Escherichia coli</i>	AMX, CFX/LEX, CIP, COT, IMI/MEM	5	MDR	21 (91.3)	AZM, GEN
<i>Klebsiella pneumoniae</i>	AMX, CFX/LEX, COT, IMI/MEM	4	MDR	11 (84.6)	AZM, CIP, GEN
<i>Enterobacter aerogenes</i>	AMX, CFX/LEX, IMI/ MEM	3	MDR	22 (88.0)	AZM, CIP, COT, GEN
<i>Proteus vulgaris</i>	AMX, CFX/LEX, COT, IMI/MEM	4	MDR	11 (92.0)	AZM, CIP, GEN
<i>Salmonella</i> species	AMX, AZM, CFX/LEX, CIP, COT, IMI/MEM	6	MDR	2 (100.0)	GEN
<i>Acinetobacter baumannii</i>	AMX, CFX/LEX, CIP, COT, IMI/MEM	5	MDR	1 (100.0)	AZM, GEN
<i>Pseudomonas aeruginosa</i>	AMX, AZM, CFX/LEX, CIP, COT, IMI/MEM	6	MDR	2 (100.0)	GEN
<i>Staphylococcus aureus</i>	AMX, AZM, CFX/LEX, CIP, COT, IMI/MEM	6	MDR	9 (100.0)	GEN, VAN
<i>Enterococcus faecalis</i>	CFX/ LEX, COT, IMI/MEM	3	MDR	9 (81.8)	AMX, AZM, CIP, GEN, VAN
<i>Streptococcus</i> species	LEX, IMI/MEM	2	Not MDR	12 (80.0)	AMX, AZM, CIP, COT, GEN, VAN

AMX: Amoxicillin, AZM: Azithromycin, CFM: Cefixime, LEX: Cephalixin, CIP: Ciprofloxacin, COT: Co-trimoxazole, GEN: Gentamicin, IMP: Imipenem, MEM: Meropenem, VAN: Vancomycin. MDR: Resistance to ≥ 3 antibiotics from different antimicrobial classes

Multidrug resistance patterns of isolates:

Of 113 bacterial isolates, 100 (91.8%) were resistant to three or more antibiotic classes, thereby meeting the definition of multidrug resistance (MDR). MDR was most frequently observed in *S. aureus* (100.0%, n=9/9), *Salmonella* species (100%, n=2/2), *P. aeruginosa* (100.0%, n=2/2) and *A. baumannii* (100.0%, n=1/1). It is noteworthy that all these bacterial pathogens are among the WHO bacterial priority pathogens (Table 4).

Multiple antibiotic resistance index of isolates:

The term MAR index gives insight into the contamination risk in the selected hospitals as well as level of antibiotic pressure. All isolates showed MAR index values exceeding the high-risk threshold of 0.2 (Table 5). *Salmonella* spp had the highest MAR index (0.85), while *K. pneumoniae* had the lowest (0.40). The mean MAR index of the isolates is 0.594 (Table 5).

Table 5: Multiple antibiotic resistance (MAR) indices of bacterial pathogens from clinical samples in three selected hospitals in Katsina State, Nigeria

Bacterial pathogen	Number of isolates	No of antibiotic tested	MAR index
<i>Salmonella</i> species	2	10	0.85
<i>Pseudomonas aeruginosa</i>	2	10	0.80
<i>Acinetobacter baumannii</i>	1	10	0.70
<i>Staphylococcus aureus</i>	9	10	0.67
<i>Proteus vulgaris</i>	12	10	0.57
<i>Enterococcus faecalis</i>	11	10	0.56
<i>Escherichia coli</i>	23	10	0.51
<i>Klebsiella (Enterobacter) aerogenes</i>	15	10	0.45
<i>Streptococcus</i> species	15	10	0.43
<i>Klebsiella pneumoniae</i>	13	10	0.40

MAR Index = Number of antibiotics to which isolate is resistant divided by the total number of antibiotics tested

Table 6: Distribution of WHO Bacterial Priority Pathogens isolated from three selected health facilities in Katsina State, Nigeria

Priority bacterial pathogen	Number	Percentage
3 rd generation cephalosporin-resistant Enterobacteriaceae	45	39.8
Carbapenem-resistant Enterobacteriaceae	23	20.4
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	9	8.0
Macrolide-resistant <i>Streptococcus</i> spp	4	3.5
Fluoroquinolone-resistant <i>Salmonella</i> spp	2	1.8
Carbapenem-resistant <i>Pseudomonas aeruginosa</i> (CRPA)	2	1.8
Penicillin-resistant <i>Streptococcus</i> spp	1	0.9
Carbapenem-resistant <i>Acinetobacter baumannii</i> (CRAB)	1	0.9
Non-priority bacterial pathogens	26	23.0
Total	113	100.0

Frequency distribution of the WHO bacterial priority pathogens:

The distribution of WHO bacterial priority pathogens identified in the study is presented in Table 6. The third-generation cephalosporin-resistant Enterobacteriaceae was the most commonly identified priority pathogen with 45 isolates (39.8%), followed by carbapenem-resistant Enterobacteriaceae with 23 isolates (20.4%). Also, 9 isolates (8.0%) were MRSA. Ampicillin-resistant *Enterococcus faecalis* and carbapenem resistant *A. baumannii* (CRAB) were the least common priority pathogens (one isolate of each, 0.9%). Twenty-six isolates (23.0%) were not listed in the WHO priority list.

According to the WHO 2024 classification of bacteria pathogens based on priority, the critical priority group constituted the largest proportion of isolates in this study, accounting for 69 isolates (79.3%), and the lowest frequency was medium priority group, with 5 isolates (5.7%) (Table 6).

Discussion:

The detection of bacterial growth in 113 of 230 clinical specimens (49.1%) in this study indicates a significant burden of bacterial infections among patients visiting the selected hospitals in Katsina State, Nigeria. Other hospital-based studies have reported similar culture positivity rates in Nigeria, indicating that bacterial infections remain widespread in healthcare facilities across most low- and middle-income countries (19,20). These findings underscore the importance of routine laboratory diagnosis in the successful management of infectious diseases.

The results also indicated that Gram-negative bacteria were more commonly isolated than Gram-positive bacteria, a trend that aligns with results from several studies conducted in Nigeria and other African countries, where Gram negative bacteria pathogens were identified as the major causes of both community- and hospital-acquired infections (21,22). The high prevalence of Gram-negative bacteria isolates can be partially attributed to their ability to survive in hospital settings and to a variety of mechanisms that allow them to resist antimicrobial agents.

Enterobacter aerogenes (formerly known as *Klebsiella aerogenes*) was the most common bacterial species identified in the study, closely followed by *E. coli*. These pathogens are widely recognized as important causes of urinary tract infections, bloodstream infections, and gastrointestinal diseases. The high occurrence of *E. coli* in urine samples observed in this study is

consistent with previous reports from Nigerian hospitals, where *E. coli* has been identified as the predominant pathogen associated with urinary tract infections (19,23). Although the occurrence of *A. baumannii* was lower, it is a clinically important pathogen due to its classification as a critical priority pathogen and known to cause severe hospital-acquired infections associated with very high mortality rates (24,25).

A microanalysis of the specimen types indicated that the largest number of bacterial isolates was obtained from urine samples, implying that urinary tract infections are a common clinical problem in the study area. The same has been reported in other studies conducted in Nigerian hospitals, where the highest frequency of bacterial isolates was consistently detected in urine samples (26). Several isolates were also recovered from stool samples, which could indicate the enteric infection burden in the area. Poor sanitation and water quality might be the causes of this trend.

The results of blood culture showed that the most common organisms associated with blood infections were *K. pneumoniae* and *E. coli*. These pathogens have often been involved in sepsis, and associated with high morbidity and mortality, especially in hospitalized patients (22). The isolation of these bacteria from blood samples underscores the severity of bacterial infections encountered in the healthcare facilities included in this study.

The antimicrobial susceptibility test results showed high levels of resistance to antibiotics such as cephalexin, imipenem and meropenem. The high resistance to carbapenems is especially alarming because carbapenems are commonly viewed as the 'last-resort' treatment options for infections caused by multidrug-resistant organisms (25,27). These levels of resistance indicate that there are increasingly more obstacles to the treatment of severe bacterial infections in the study areas. The high resistance levels observed in this study are comparable to findings reported in other regions of Nigeria, where inappropriate use of antibiotics is a primary driver of antimicrobial resistance (26,28). Self-medication, over-the-counter availability of antibiotics, and empirical treatments are likely contributing factors to the high resistance levels observed.

Although resistance levels to several antibiotics by the isolates were high, resistance to gentamicin and vancomycin (by Gram-positive bacteria) were relatively low, which implies that these antibiotics may still be effective against certain infections in the study area. Similar results have been reported in prior Nigerian res-

earch, in which gentamicin retained significant activity against both Gram-positive and Gram-negative pathogens (21). Nonetheless, continued use of a few effective antibiotics may ultimately lead to increased resistance if antimicrobial stewardship programs are not established. Another important finding of this study was the high MAR index values observed among the isolates, all greater than 0.2. These values are typically regarded as indicators of bacterial strains that have evolved in environments with high antibiotic exposure. This has also been observed in hospitals, where antibiotic misuse and over-use contribute to intense selection on microbial populations (19).

The overall prevalence of MDR in the study was notably high. Several bacterial species, including *S. aureus*, *Salmonella* species, *P. aeruginosa* and *A. baumannii*, were 100% MDR. This observation is consistent with findings from earlier Nigerian studies that reported high rates of MDR among bacterial pathogens categorized as WHO priority organisms (22,29). The presence of MDR bacterial pathogens poses a serious challenge to clinical management because it significantly reduces available treatment options and may lead to prolonged hospital stays and increased treatment costs. Consistent with the study objective of identifying WHO bacterial priority pathogens, a large proportion of the isolates detected belonged to the critical priority category, particularly third-generation cephalosporin-resistant and carbapenem-resistant Enterobacteriaceae. These are also consistent with the reports provided at the global and national level that consider Enterobacteriaceae among the key factors that increase the burden of AMR (2,30).

Moreover, the detection of MRSA in the study confirms earlier findings that MRSA infections are very common in healthcare centers in Nigeria (29). The presence of pathogens belonging to the medium priority category was less common, but they also pose a significant risk of disease and, especially, can be detrimental to vulnerable groups, like children, older people, as well as immunocompromised patients (31).

There are some limitations in this study. First, this study was conducted in only three hospitals in Katsina State, Nigeria, therefore, the findings may not fully represent the situation in all healthcare facilities within the State or Nigeria as a whole. In addition, bacterial identification and resistance detection were based mainly on phenotypic laboratory methods, and molecular techniques were not used to confirm carriage of resistance genes by the isolates.

Conclusion:

The findings of this study indicate that

WHO priority bacterial pathogens are common in hospital settings in Katsina State, Nigeria. Gram-negative bacteria were the most frequent pathogens identified, with *K. aerogenes* and *E. coli* being the most common bacterial species from the clinical samples tested.

The study also showed that most bacterial isolates exhibited high resistance, with some resistant to antibiotics used as last-line treatments for serious bacterial infections. Moreover, high frequency of the isolates was MDR, underscoring the increasing difficulty of treating bacterial infections in the study area. These results underline the necessity to intensify antimicrobial stewardship initiatives, routine laboratory diagnosis and infection prevention and control measures in healthcare facilities to contain the proliferation of resistant bacterial pathogens.

Contributions of authors:

SHA, MGL and HMR were involved in the study conceptualization, data collection, laboratory analysis, data interpretation, manuscript drafting and critical revision of the manuscript. All authors approved the final version submitted for publication.

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Authors declare no conflicts of interest.

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