





# Mapping Antibiotic Resistance Genes in Bacteria of Medical Interest and the Evolution of Healthcare-Associated Infections in Chad: 2012 to 2025

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## Abstract

Multidrug-resistant (MDR), highly resistant (XDR), and pan-resistant (PDR) bacteria to antimicrobials are a major public health problem worldwide, particularly in America, Africa, Europe, Russia, and Chad, due to their prevalence, impacts, difficulties in therapeutic management, and socio-economic consequences. This was a descriptive, cross-sectional, and etiological diagnostic study conducted from January 17, 2012, to December 7, 2025, at the bacteriology and mycobacteria laboratories of the National Reference Hospital Center (CHURN) of N'Djamena, and at the Bacteriology Unit of the Laboratory for Research, Diagnostic and Scientific Expertise (Labo-ReDES) of the Faculty of Human Health Sciences (FSSH) of the University of N'Djamena, following standard phenotypic and molecular methods of clinical microbiol-

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ogy. The objective of this study was to produce a map of resistance genes of MDR, XDR, and PDR to antimicrobials and healthcare-associated infections from 2012 to 2025 in Chad. In the study, 851 resistant bacterial phenotypes were identified, including 329 MDR bacteria, 407 ESBL, 114 XDR bacteria, and 10 PDR. The emerging resistant bacteria identified were: *Staphylococcus aureus* (65%), *Escherichia coli* (90%), *Klebsiella pneumoniae* (25%), *Enterobacter cloacae* (8%), *Salmonella* spp. (18%), *Acinetobacter baumannii* (2%), and *Shigella flexneri* (21%). The identified CREs were: *Enterococcus* (11%) and carbapenemase-producing Enterobacteriaceae (*Escherichia coli*, *Enterobacter*, *Serratia*, etc.). *Vibrio cholerae* (25%) and *Neisseria meningitidis* (34%) were also present. The detected resistance genes were those of: *Mycobacterium tuberculosis* (*rpoB*, *inhA*, *KatG*/*inhA*, *gyrA* and *gyrB*, *rrs* and *eis*), *Mycobacterium leprae* (*rpoB*, *floP1*, *gyrA* and *gyrB*), and the chloride toxin-producing genes of *Vibrio cholerae* (*ctxA*, *ctxB*, *gyrA*, *gyrB*), *Staphylococcus* and Enterobacteriaceae (*E. coli*, *Klebsiella*, *Enterobacter*, etc.: *mecA*, *ACC* (6') and *Enterococcus* (*vanA*). The results of this study highlight the importance of mandating adherence to antituberculosis and antileprosy medications for patients, requiring prescribers to prescribe based on laboratory evidence, and ultimately, urging policymakers to organize laboratories in the country's 23 provinces and equip them with high-performance diagnostic tools. The data from this study should also encourage national programs in Chad, Africa, and around the world to activate the BMR, XDR, and PDR surveillance system.

## Keywords

Mapping, Resistance Gene, MDR, PDR, XDR, Antimicrobial, Healthcare-Associated Infections, Chad

## 1. Introduction

Multidrug-resistant (MDR), highly resistant (XDR), and pan-resistant (PDR) bacteria are a major public health problem worldwide. These bacteria pose a risk of therapeutic impasse, necessitating broad-spectrum probabilistic antibiotic therapy, which is responsible for new bacterial selections [1] [2]. The production and dissemination of hundreds of thousands of tons of antibiotics, for all uses combined (human and veterinary medicine, agriculture), have constituted a new stress for half a century, which the bacterial world has faced without too much difficulty [3] [4]. This is particularly the case for  $\beta$ -lactams, which are the mainstay of antibiotic therapy for Enterobacteriaceae infections. These resistances are the phenotypic expression of genetic modifications appearing at the time of mutations or transfers of genetic information in certain potentially very pathogenic Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Enterobacter aerogenes*, etc.), which are resistant to all molecules of this class, including carbapenems [5] [6]. These strains are often co-resistant to many other antibiotics, which can make treatment very problematic. Infections caused by multidrug-resistant MDR, XDR, and PDR bacteria are associated with high morbidity and

mortality, prolonged hospital stays, and increased hospitalization costs [7]. These bacteria can be transmitted to other patients through the hands of healthcare workers or a contaminated environment. The resistance rate of various infectious agents varies across geographic areas and countries, but everywhere it has significant public health implications [8].

Resistance genes can be divided into the following categories according to the class of antibiotics to which they confer resistance, including tetracyclines (tet), sulfonamides (sul),  $\beta$ -lactams (*bla*, *mecA* etc.), macrolides (*erm*), aminoglycosides (aac), fluoroquinolones (*fca*, *gyr*), colistin (*mcr*), vancomycin (*van*), multidrug resistance (*mdr*), antituberculosis and antileprosy drugs (*rpoB*, *inhA*, *rrs*, *eis*, *flopI*) [9]-[13]. Chad also faces the challenge of multidrug-resistant bacteria (MDRB) and highly resistant bacteria (HReB), which pose a major threat to the country's public health, given the increasing prevalence of these bacteria in recent years. Data on antibiotic resistance are therefore very limited in Chad because bacterial isolation and antibiogram testing are rarely requested by prescribers. With the aim of producing documentation on BMR, XDR, PDR and genetic mutations associated with drug resistance in hospital and community settings, this study was envisaged over a period from January 7, 2012 to December 17, 2025 in Chad.

## 2. Materials and Methods

### 2.1. Framework, Period, and Type of Study

This was an analytical, descriptive, cross-sectional, and etiological diagnostic study conducted on resistant, multidrug-resistant, and extensively resistant bacteria from 07/01/2012 to 17/12/2025, which was conducted and executed at or at:

- Bacteriology Unit and Laboratory Mycobacteria Unit of the National Reference Hospital Center (CHURN) of N'Djamena;
- Bacteriology Unit of the Research, Diagnostic, and Scientific Expertise Laboratory (Labo-ReDES) of the Faculty of Human Health Sciences (FSSH) of the University of N'Djamena;
- Pasteur Institute of Paris and the laboratory of the University Hospital of Montpellier (France);
- University Center for Clinical Research (CURC), University of Sciences, Techniques, and Technologies of Bamako (USTTB), Bamako, Mali.

### 2.2. Eligibility Criteria

#### - Inclusion Criteria

Any consenting individual in community or hospital settings who presents with symptoms of infectious diseases (acute carriers) or is asymptomatic (chronic carriers) and has benefited from diagnostic tests related to the objective of the study.

#### - Exclusion Criteria

The following items are not included in this study:

- Any individual who has not undergone diagnostic testing for the main biological markers used to assess infectious disease control;

- Patients/individuals who do not consent to participate in this study.

### 2.3. Sampling

Convenience sampling was used in relation to the duration of the study.

Samples of pathological biological products were collected from patients in community and hospital settings in different localities of Chad throughout the study period.

### 2.4. Choice of Anti-Infectives

Antibiotics were chosen based on their prescription for the treatment of various infectious diseases (**Table 1**) [14]. Antituberculosis drugs (1st line: rifampicin, isoniazid; and second line: levofloxacin, amikacin, kanamycin, and capreomycin) and antileprosy drugs (1st line: dapson, rifampicin, and clofazimine; and second line: ofloxacin, minocycline, and clarithromycin) were administered according to the management guidelines recommended to national tuberculosis and leprosy control programs.

**Table 1.** Antibiotics chosen for the susceptibility test.

Category	Family	Antibiotic/load	Diameter (mm)		
			Sensitivity	Intermediate	Resistance
Antibiotics (Bio-Rad)	Aminosides	Gentamicin (10 µg)	>16	14 - 16	<14
		Penicillin G (6 µg)			
		Methicillin (5 µg)			
	B-Lactamines	Oxacillin (5 µg)	>ou = 20		
		Ampicillin (10 µg)	>14	12 - 14	<12
		Amoxicillin (25 µg)	>23	19 - 23	<19
		AMC (20 - 10 µg)	>24	18 - 24	<18
		Aztreonam (30 µg)	>24	19 - 25	<19
		Cefotaxime (30 µg)	>31	25 - 31	<25
		Cefepime (30 µg)	>22	19 - 22	<19
		Imipenem (10 µg)	>32	17 - 24	<17
	Cyclins	Tetracycline (30 µg)	>20	17 - 20	<17
		Doxycycline (30 µg)	>20	17 - 20	
	Fluoroquinolones	Ciprofloxacin (5µg)	>22	19 - 22	<19
	Quinolones	NAL (30 µg)	>22	19 - 22	<19
Sulfamides	SXT (1.25/23.75 µg)	>22	19 - 22	<19	
Glycopeptides	Vancomycin (30 µg)	>ou = 17			
Macrolides	Erythromycin (15 U I)	>26	24 - 26	<24	

Quality control was performed using the reference strain *E. coli* ATCC 25922. Legend: Nalidixic acid (NAL); trimethoprim-sulfamethoxazole (SXT). Susceptible = Treatment success; Resistant = Treatment failure; Intermediate = Uncertain.

## 2.5. Isolation of Bacteria

The isolation of bacteria was carried out in different agar media: Hektoen (all enterobacteria), Chapman (staphylococci), chocolate agar with cooked blood or fresh blood (streptococci), TCBS agar (vibrios), Sabouraud chloramphenicol agar (molds and yeasts), etc.

## 2.6. Biochemical Identification of Bacteria

The galleries: API20E, STREP, STAPH, etc., were used for the biochemical identification of the isolated bacteria. The MYCOPLASMA IST 3 gallery (BioMérieux, Marcy l'Etoile, France) enabled the biochemical identification and antibiogram testing of *Mycoplasma hominis* and *Ureaplasma* spp. Six (6) antibiotics are incorporated into the gallery; five (levofloxacin, moxifloxacin, tetracycline, erythromycin, and telithromycin) were tested against *Ureaplasma*, and four (clindamycin, levofloxacin, moxifloxacin, and tetracycline) were tested against *Mycoplasma hominis*.

## 2.7. Detection of Multidrug-Resistant and Ultra-Resistant Bacteria Using the Kirby-Bauer Technique

The sensitivity test method used is the diffusion method in MH agar (disc method or Kirby-Bauer technique) following the recommendations of the Antibiogram Committee of the French Society of Microbiology and the European Committee on Antimicrobial Susceptibility Testing, the Clinical Laboratory Standards Institute (CLSI), and the Food and Drug Administration (FDA) of the United States of America [15] [16].

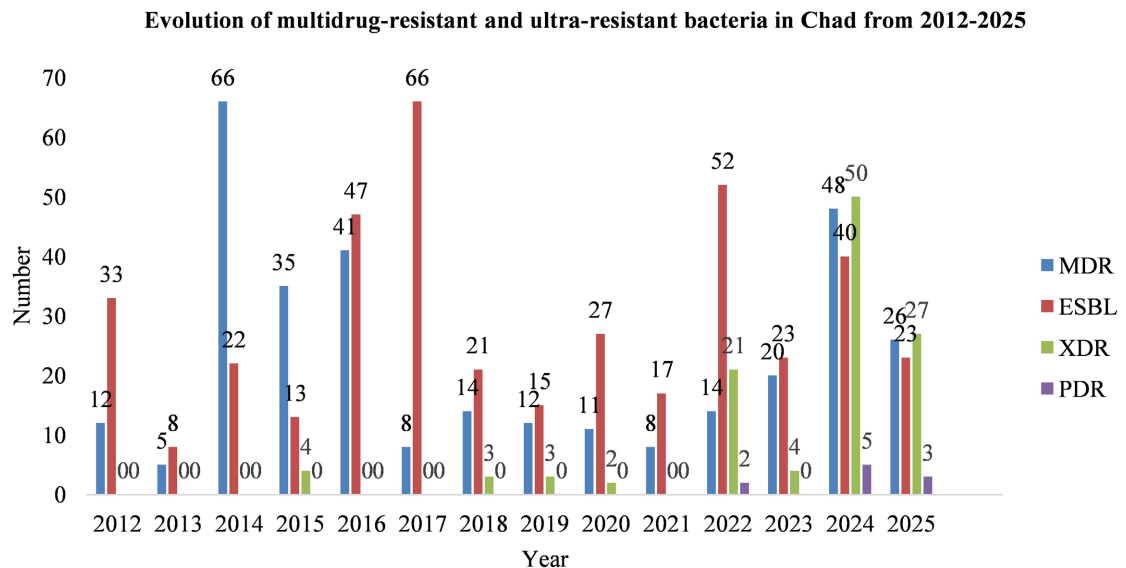
## 2.8. Detection of Extended-Spectrum Beta-Lactamase (ESBL) Production

ESBL production was detected on Muller-Hinton agar using the double-disc synergy assay. Discs of cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), and aztreonam (30 µg) were placed 30 mm (center to center) from an amoxicillin/clavulanic acid (20/10 µg) disc and then incubated at 35°C - 37°C (Figure 1). After 18 - 24 hours of incubation, ESBL production by the test organism was determined based on the partial inhibition of ESBL by clavulanic acid. The existence of even a weak synergy between cefotaxime, ceftazidime, cefepime, aztreonam, and clavulanic acid is characterized by a champagne cork-shaped image [17].

## 2.9. of Resistance Genes, Multidrug-Resistant Bacteria, and Emerging Highly Resistant Bacteria by Vitek Compact 60

The Vitek Compact 60 was also used to detect resistance phenotypes in isolated bacteria. The system includes the Vitek® 2 Compact instrument, a computer (workstation), and a printer. The software provided with the Vitek® 2 Compact system includes data analysis and management programs. A bidirectional computer interface automatically transfers results to the user's Laboratory Infor-

mation System (LIS) and to various product and patient reports. A quality control system is available to validate a Vitek® 2 Compact system test kit. An Advanced Expert System™ (AES) (for clinical use) is available to allow systematic online validation of results and interpretation of resistance phenotypes identified by antibiograms. The Vitek 2 range allows for same-day access to the information needed to initiate targeted antibiotic therapy, thus ensuring better patient care and cost savings in healthcare. Vitek has also been used to identify the genes (*mecA*, *ACC(6')* and *vanA*).



**Figure 1.** Evolution of multidrug-resistant and extensively drug-resistant bacteria associated with healthcare in Chad from 2012-2025.

### 2.10. Detection of the *ctxB* and *ctxA* Genes Using Molecular Methods at the Pasteur Institute in Paris (France)

Molecular characterization of the isolated *V. cholerae* O 1 strains was performed at the National Reference Center for *Vibrio* and Cholera (CNRVC) of the Pasteur Institute in Paris, France (IPP/F). The procedures were carried out according to the CNRVC's instructions at the IPP/F. PCR testing for pathogenicity factors was systematically applied to all *V. cholerae* O 1 strains sent to the CNRVC at the IPP/F. PCR was also used to confirm the *V. cholerae* O 1 strains from Chad. After detection of the PCR product on a 2% agarose gel, the electrophoretic migration program was as follows: initial pulse time 60 s, final pulse time 120 s, migration time 72 hours at 6 V/cm<sup>2</sup>, angle 120°, temperature 14°C. At the end of the migration, the gel was stained with ethidium bromide (ET, 1.5%) for 30 min. Visualization was performed under a UV lamp with an imager, incorporating IPP/F software.

### 2.11. Detection of Hepatitis A, B, C, and E Viruses

Rapid immunochromatographic tests were used to detect hepatitis A, B, C, and E

viruses, as well as dengue viruses and non-culturable bacteria. The tests were performed according to the manufacturer's instructions.

### 2.12. Detection of Yellow Fever Virus

The CDC MAC-ELISA 72 test at the Laboratory of the National Reference University Hospital Center (LCHU-RN) in N'Djamena was used for the detection of the yellow fever virus. Samples collected from the various provinces were received, sorted, prepared (aliquoted), and stored in a cold room ( $-80^{\circ}\text{C}$ ) at the Virology Unit of the National Reference University Hospital Center in N'Djamena for analysis.

### 2.13. Detection of *Plasmodium falciparum*

Thick blood film and thin blood film, as well as rapid detection tests, were used to detect *Plasmodium falciparum*.

### 2.14. Detection of Tuberculosis Using Three Molecular Techniques in Chad

The following molecular techniques have been used to detect resistance genes in order to map them [18]:

#### 1) Tuberculosis Detection Using the TB-LAMP Automated System

TB-LAMP is a molecular diagnostic technique that has enabled sensitive and accurate detection of tuberculosis. It is easy to use and provides reliable results within one hour. The process consists of four steps:

- Sputum preparation: 60  $\mu\text{L}$  of sputum is transferred to the heating tube, stirred to mix well, and incubated in the HumaLoop T heating unit at  $90^{\circ}\text{C}$  for 5 minutes;
- DNA extraction: The pretreated (lysed) sample in the heating tube was transferred to the absorbent tube containing absorbent powder, which eliminates any potential inhibitors of the LAMP reaction. The purified DNA was extracted and directly transferred to the LAMP reaction tube.
- Amplification: The DNA transferred to the LAMP reaction tube was incubated for 2 minutes at room temperature to reconstitute the reagents in the cap. It was then homogenized several times and tapped until the reaction mixture settled at the bottom of the tube. The reaction tube was then transferred to the HumaLoop T reaction unit at  $67^{\circ}\text{C}$  for 45 minutes.

#### Reading the results

The reaction tube was finally inserted into the UV lamp detection unit, and the lamp was switched on.

- A positive result produces a green light;
- A negative result produces no fluorescence.

Real-time turbidity readings and automatic reporting of results.

#### 2) Detection of rifampicin sensitivity and resistance using the GeneXpert analyzer

**Principle:** It is based on amplification of a fragment of the *rpoB* gene containing

the central region of 81 base pairs and fragments of the target sequences of the IS1081 and IS6110 insertion elements with multiple copies by primers.

Using a Pasteur pipette, 2 mL of sputum and 4 mL of the reagent were collected and mixed in another sterile jar. The mixture was vortexed and incubated at room temperature for 10 minutes. The jar was vortexed again and incubated at room temperature for 5 minutes. Then, 2 mL of the liquefied mixture was aspirated and transferred to the Xpert<sup>®</sup> MTB/RIF ULTRA cartridge, and the test was launched for a duration of 1 hour and 30 minutes.

### **3) Detection of rifampicin and isoniazid resistance genes by the automated Line Probe Assay (Hain)**

**Principle:** It is based on DNA-STRIP technology and includes DNA extraction, master mix preparation, multiplex amplification with biotinylated primers, and detection by reverse hybridization.

Sputum samples were decontaminated using the BD BBL<sup>™</sup> MycoPrep kit containing N-Acetyl L-Cysteine-sodium hydroxide and phosphate buffer. DNA was extracted from the samples using the GenoLyse kit (Hain Life Science). 500 µL of the decontaminated sample was taken and transferred to Eppendorf tubes and centrifuged at 10,000 rpm for 15 minutes. After centrifugation, only the pellet was kept, to which 100 µL of lysis buffer solution (A-LYS) was added, mixed by vortexing, and incubated for 5 minutes at 95°C in a water bath. Afterwards, we added 100 µL of neutralization buffer (A-NB), then vortexed the mixture for 5 seconds and centrifuged quickly for 5 minutes in order to bring the DNA from the pellet to the supernatant. Our interest was focused on the supernatant. The extracted DNA was then amplified by PCR and to do this, we prepared 45 µL of mixture (10 µL of MixA + 35 µL of MixB) for each sample, then introduced 45 µL of mixture (MixA + MixB) into each tube. Then, 5 µL of the DNA from each sample was added. After amplification, the PCR products (amplicons) were hybridized with the probes fixed on the strip. To do this, 20 µL of denaturation solution (DEN, blue) was distributed in the corner of each of the wells used, to which 20 µL of amplified DNA was added. The mixture was then homogenized and incubated at room temperature for 5 minutes. 1 mL of preheated hybridization buffer was added to each well and then the tray was gently shaken until the solution had a homogeneous color. Subsequently, a strip was placed in each well. We placed the tray in the shaking water bath/TwinCubator and incubated it for 30 minutes at 45°C. We then aspirated the hybridization buffer completely and added 1 mL of stringent wash solution to each strip, incubating for 15 minutes at 45°C in a shaking water bath/TwinCubator. The stringent wash solution was then completely vacuumed up. Each strip was washed once with 1 mL of rinse solution for 1 minute on the shaking platform/TwinCubator (pour out the RIN after incubation), then 1 mL of diluted conjugate was added to each strip and incubated for 30 minutes on the shaking platform/TwinCubator. The solution was removed, then each strip was washed twice for 1 minute with 1 mL of rinsing solution and once for 1 minute with 1 mL of distilled water on a shaking platform/TwinCubator

(pour the solution off each time), then 1 mL of diluted substrate was added to each well, incubated away from light for 5 minutes, rinsed briefly twice with distilled water, and the strips were dried.

Line Probe Assay (LPA) or reverse hybridization strip tests are DNA strip tests which make it possible to determine the drug resistance profile of a strain of the *Mycobacterium tuberculosis* Complex through the pattern of amplicon binding (amplification products DNA) to probes targeting the mutations most often associated with resistance of the *Mycobacterium tuberculosis* Complex to first- and second-line anti-tuberculosis drugs, and to probes targeting the corresponding wild-type DNA sequence.

#### **4) Detection of mycobacteria by the spoligotyping technique [19]**

Culture and spoligotyping were performed at the University Clinical Research Center (CURC), University of Sciences, Techniques and Technologies of Bamako (USTTB), Bamako, Mali. All culture-positive samples were used for spoligotyping. Spoligotyping was performed on boiled bacterial lysates using a commercially available kit (Ocimum) or an internally prepared membrane. In short, this technique amplifies the polymorphic region called DR (Direct Repeat) using two external primers (DRa and DRb). We performed real-time PCR for amplification. Hybridization was then carried out on an Isogen membrane. Development was performed using a streptavidin conjugate and the ECL (Enhanced Chemi-Luminescence) detection kit. A film strip was used on the Isogen membrane in a dark chamber for development and detection. The results were recorded in octal code on the film strip, and strain comparisons were performed with SPOTCLUST (based on SpolDB3) and SITVIT2 (available at <http://www.pasteur-guadeloupe.fr:8081/SITVIT2>). This database is currently a major genotypic marker database with over 111,635 genotypes isolated from 163 countries. Approximately 174 isolates were used for spoligotyping, the results of which are also recorded in octal code.

### **2.15. Detection of Mycobacterium Leprae Antimicrobial Resistance Genes by PCR-RLEP Amplification**

DNA amplification by PCR targeting RLEP repeat sequences was performed as described by biologists at the Arnaud de Villeneuve University Hospital in Montpellier, France. After detection of the PCR product on a 2% agarose gel, the amplicons were processed according to the Sanger sequencing tube protocol [20].

### **2.16. Data Processing**

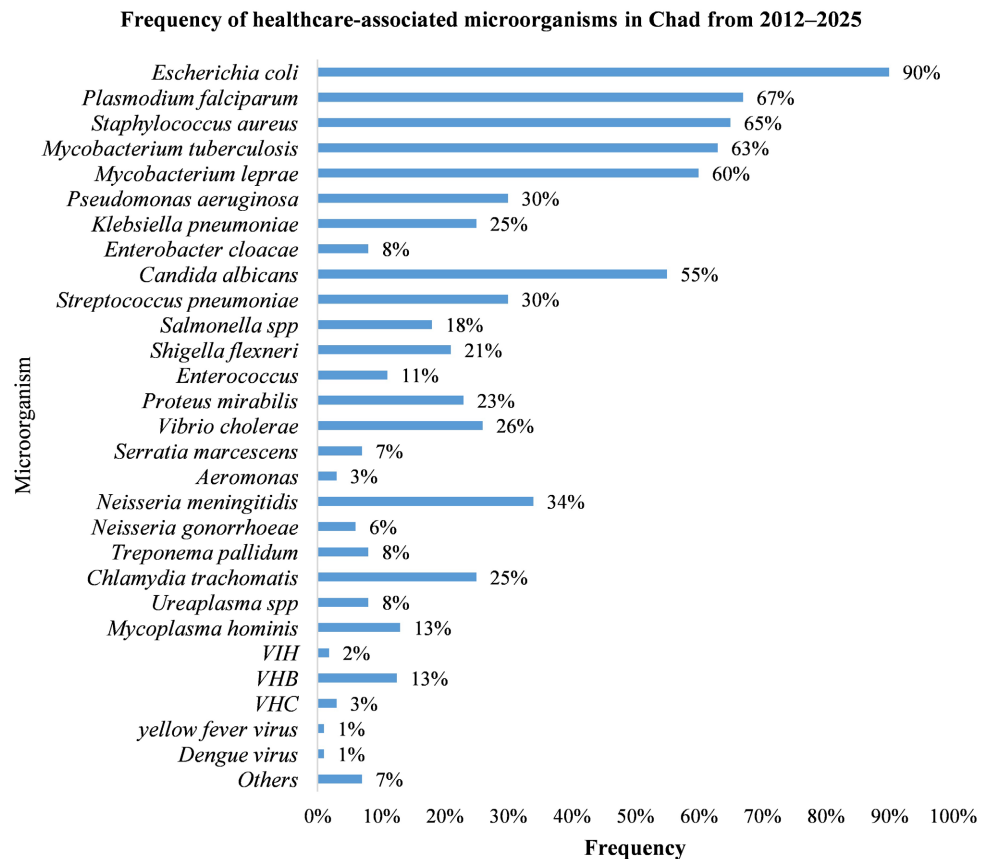
Results, including control data for key biological markers and data from information forms, were entered into a spreadsheet using MS-Excel. Descriptive analysis consisted of presenting the collected data as counts, percentages, means, and graphics. GPS Maps was used to map and locate the MDRs and resistance genes on a map of Chad.

### 3. Results

#### 3.1. Frequency of Healthcare-Associated Microorganisms

Healthcare-associated infections were dominated by the bacterium *Escherichia coli* (90%), followed by infections from blood-sucking parasites (*Plasmodium falciparum*; 67%) and fungal agents such as *Candida albicans* (55%).

The investigation of microorganisms associated with female and male infertility revealed 30.37% *Chlamydia trachomatis* detected by immunochromatographic tests, 18 (13.33%) *Mycoplasma hominis*, and 11 (8.14%) *Ureaplasma* spp. identified by biochemical tests (API-MYCOPLASMA IST 3 with antibiogram test) (Figure 2).



**Figure 2.** Frequency of healthcare-associated microorganisms in Chad: 2012-2025.

#### 3.2. Evolution of Multidrug-Resistant and Extensively Drug-Resistant Bacteria Associated with Healthcare in Chad from 2012-2025

The multi-resistant bacteria detected were distributed according to the biochemical mechanisms of resistance, either to beta-lactams ( $\beta$ -lactams) by production of extended-spectrum beta-lactamases (ESBL), by production of acquired penicillinases, or by modification of penicillin-binding proteins (PBPs) induced by resistance genes. During 24 years (2012-2025) of study, 851 resistant bacterial phe-

notypes were identified, including 329 MDR (bacterial isolates that remain resistant to at least three or more classes of antibiotics), 407 ESBL (Extended Spectrum Beta-lactamases), 114 XDR (bacterial isolates that remain sensitive to antibiotics in only one or two classes), and 10 PDR (bacterial isolates that remain resistant to all classes of antibiotics tested locally). Among the 10 PDRs, there were 3 *Pseudomonas aeruginosa*, 3 *Escherichia coli*, 2 *Proteus* spp., and 2 *Klebsiella pneumoniae*. The main multidrug-resistant and extensively drug-resistant bacteria identified were named with their bacterial name and the name of the antibiotic to which they were resistant: methicillin-resistant *Staphylococcus aureus* (MRSA), VRE (Vancomycin-resistant *Enterococcus*), and Extended Spectrum Beta-Lactamases (ESBL) producing Enterobacteriaceae. The most frequently reported resistant bacteria (MRB) were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*, followed by *Salmonella* spp. Among the identified MDR bacteria, 48 (11%) of the *Mycobacterium leprae* strains were detected positive by PCR-RLEP, the sequencing of which revealed two mutant strains resistant to rifampicin and ofloxacin, with a mutation rate of 1.2%. Molecular techniques (GeneXpert, TBLAMP, Line Probe Assay) detected 66 (15%) *Mycobacterium tuberculosis*-positive (MTB+) strains that were resistant to first-line antituberculosis drugs. Of these 66 MTB+ strains, 34 (13%) were resistant to rifampicin, 10 (4%) were resistant to isoniazid, and 22 (8%) were multidrug-resistant.

Conventional culture allowed the isolation of 329 bacteria commonly responsible for infections in hospital and community settings, the results of which revealed the following:

- From 1971 to 2025, Chad experienced 45 cholera epidemics, with 86,961 cases and 6567 deaths (7.55%). The spread of the disease was linked to sociocultural practices in Chad and neighboring countries, particularly those related to water systems. The first *Vibrio cholerae* O1 serotype Ogawa was isolated in 1971, and the last isolates, found during the 2025 epidemic in refugee camps in the east, were all susceptible to doxycycline. The susceptibility profiles of the strains (*Vibrio cholerae* O1, serotype Ogawa, biotype El Tor, and classical) to other classes of antibiotics varied. Resistance rates of 100% were observed with trimethoprim-sulfamethoxazole, nalidixic acid, and the vibriostatic compound O/129. The second most active antibiotics were tetracycline (99%), norfloxacin (78.14%), and ciprofloxacin (76%);

- Resistance frequencies were also noted in 120 isolated strains of *Staphylococcus aureus*. This study showed that the *Staphylococcus* strains were 100% resistant to methicillin, followed by 82% to vancomycin, 46% to erythromycin, 43% to gentamicin, and 42% to ciprofloxacin. Six (6) strains of *Staphylococcus aureus* were resistant to antibiotics from the following families: ampicillin/sulbactam (MIC  $\geq$  8), linezolid (MIC  $\geq$  8), and trimethoprim-sulfamethoxazole (MIC  $\geq$  320), respectively. The acquired penicillin resistance of these isolated strains was cross-resistant with the majority of beta-lactams, but at varying levels depending on the antibiotics used, allowing the use of the most active molecules. Another example

of observed cross-resistance was methicillin resistance in *Staphylococcus aureus* strains (68.33%) due to a modification of a PBP that also confers resistance to other molecules in the beta-lactam family;

- Two strains of *Klebsiella pneumoniae* detected produced associated  $\beta$ -lactamases, which hydrolyzed carbapenems moderately and other  $\beta$ -lactams weakly by the biochemical mechanism allowing the reduction of the permeability of the outer membrane;

- Three strains of *Salmonella* identified were resistant to beta-lactams by production of ESBLs and to nalidixic acid (quinolones);

- Among the strains of bacteria producing extended-spectrum cephalosporinases (ESBLs) that inactivate 3rd generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime, cefixime), 8 strains of *Escherichia coli* produced *AmpC* (cyclic adenosine monophosphate) type cephalosporinases, which is a resistance mechanism by enzymatic inactivation that increases the MIC of beta-lactam antibiotics: ceftriaxone (MIC  $\geq$  32), cefixime (MIC  $\geq$  64), cefotaxime (MIC  $\geq$  64), ceftazidime (MIC  $\geq$  6). Most of the enterococcal strains isolated in this study exhibited various multi-resistance phenotypes to antibiotics from different families;

- Twenty-one (21) BMRs have developed multi-resistance associated with the families (beta-lactams, glycopeptides, macrolides/lincosamides/ST/reptogramins), by modification of PBP induced by the *mecA* gene, or by inducible *MLSb*;

- Fourteen MDRs were resistant to the families (beta-lactams, aminoglycosides) either by production of ESBLs or by acquisition of plasmid resistance induced by the asparagine protein due to the presence of the *AAC* (6') nucleotide;

- Four strains of *Neisseria meningitidis* with reduced susceptibility to penicillins and two strains resistant to third-generation cephalosporins were detected in Goundi.

- *Ureaplasma* spp. developed an average resistance of 86.52% to fluoroquinolones, 32% to macrolides, and 36.36% to tetracycline. *Mycoplasma hominis* developed an average resistance of 36.16% to fluoroquinolones, 39% to tetracycline, and 28% to lincosamides.

The main risk factors identified in the transmission of these multidrug-resistant bacteria in hospital settings were urinary tract infections and the presence of a urinary catheter in patients hospitalized in wards.

- Finally, the study revealed the presence of 72 fungal agents (51 *Candida albicans*, 3 *Cryptococcus laurentii*, 1 *Candida krusei*, 8 *Candida lipolytica*, 4 *Aspergillus niger*, 5 *Aspergillus fumigatus*) resistant to varying degrees to antifungals (azoles (5-Fluorocytosine, Econazole, Clotrimazole, Fluconazole, Voriconazole) and polyenes (Amphotericin B, Nystatin)). The fungal strains were sensitive (83.63%) to azole derivatives and resistant (96%) to polyenes. Azole derivatives were more active than polyenes. The activity of fluconazole was limited.

### 3.3. Distribution of Resistance Genes at Study Sites

**Figure 3** illustrates the surveyed cities where resistance genes were distributed: Adré, Goz Beida, Farchana, Choukoyane, Abéché, Guera, Barh El Gazel, Hadjar-

lamis, Bol, N'Djamena, Bousso, Bongor, Kélo, Pala, Léré, Moundou, Bebedjia, Doba, Goundi, Sahr, and Goré.

Chad is located between 7° and 24° North latitude and 13° and 24° East longitude. It covers an area of 1,284,000 km<sup>2</sup> and is the fifth largest country in Africa after Sudan, Algeria, Zaire, and Libya. It stretches 1700 km from north to south and 1000 km from east to west. It shares borders with Libya to the north, Sudan to the east, the Central African Republic to the south, and Cameroon, Nigeria, and Niger to the west. The city of Abéché, located 900 km north of N'Djamena, shares borders with Libya to the north and Sudan to the east, while Goré, 600 km south of Chad, borders the Central African Republic and Cameroon to the west. Politically and economically, the country belongs to Central Africa, but due to similar climatic conditions, it is also considered part of the Sahel region. Chad's geographical location has likely contributed to the distribution of multidrug-resistant (MDR) organisms, highly resistant (XDR), pan-resistant (PDR) bacteria, and resistance genes. Among the biopsies performed for *Mycobacterium leprae* testing, 60% were positive for PCR-RLEP. Sequencing revealed two mutant strains of *Mycobacterium leprae* associated with resistance to rifampicin and ofloxacin. A missense mutation at codon 456rpoB (Ser (TCG > Leu (TTG))) was observed in a secondary case of rifampicin resistance in a 46-year-old relapsing patient from N'Djamena. Additionally, a missense mutation at codon *gyrA* 91 (Ala (GCA > Val (GTA))) associated with ofloxacin resistance was found in a 37-year-old patient from Abéché who had not been sensitized to ofloxacin but had been exposed to other quinolones. No mutations were observed in the *floP1* gene.

Of the positive cases of *Mycobacterium tuberculosis* (MTB+) detected by molecular techniques (TBLAMP and GeneXpert), 25% were resistant to first-line anti-tuberculosis drugs. Among the 25% of resistant MTB+ cases, the automated Line Probe Assay (Hain) detected 13% rifampicin-resistant genes (*rpoB*), 4% isoniazid-resistant genes (*inhA*), and 8% multidrug-resistant genes (*mdr*) (*rpoB/KatG* and/or *KatG/inhA*). Among the genes detected with antibiotic-associated mutations (Levofloxacin (Lfx), Amikacin (Am), Kanamycin (Km), and Capreomycin (Cm)) by the Line Probe Assay technique, 13.6% (*gyrA*, *gyrB*) were due to gyrase enzymes, 7.6% (*eis*), 6.1% (*rrs* and *eis*), and 10.6% were mutations affecting different alleles on the chromosomes of the *Mycobacterium tuberculosis* complex. The mechanism of resistance to Levofloxacin was manifested by point mutations occurring in specific regions of two enzymes: DNA gyrase A and DNA gyrase B.

Spoligotyping of *Mycobacterium tuberculosis* isolates resulted in 5 groups, ranging from 4 to 9 isolates per group. Of the twelve octal codes identified by the spoligotyping technique, three lineages exhibited the same genotypic characteristics as the major genotypic lineages (Euro-American, Indo-Oceanic, East-African-Indian).

The strains of *Vibrio cholerae* O 1 isolated during the successive epidemics of 2011-2012 in Chad were confirmed in 71/72 cases (98.61%) by molecular methods at the Pasteur Institute in Paris (France) as carrying the virulence genes encoding

the synthesis of cholera toxin (*ctxB* and *ctxA*).

Eight *Staphylococcus aureus* strains (6.7%) producing acquired penicillinase through modification of PBP (penicillin-binding protein) induced by the *mecA* gene were also detected. Six (6) *Staphylococcus aureus* strains resistant to beta-lactams through penicillinase production and to the oxazolidone and sulfonamide families through acquisition of plasmids induced by the *ACC(6')* gene were identified. Seventeen (17) *Staphylococcus haemolyticus* strains were identified, of which 12/120 (10%) produced second-generation cephalosporinases through modification of PBP induced by the *mecA* gene.

Ten strains of *enterococci* (*Enterococcus* (9.1%), *Enterococcus faecalis* (67%), and *Enterococcus faecium* (33%)) isolated in this study exhibited multi-resistance phenotypes to antibiotics from different families by induction of the *vanA* gene (Figure 3).

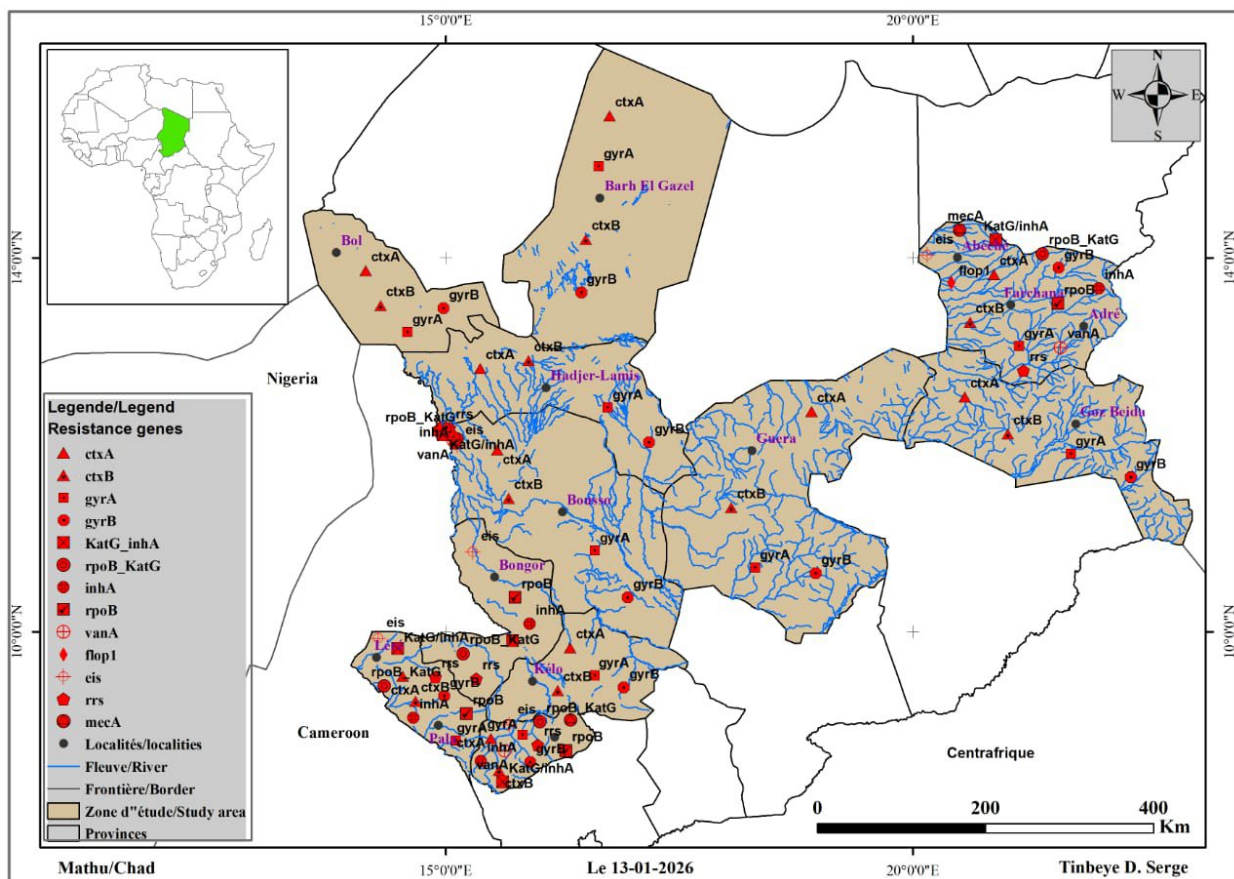


Figure 3. distribution of resistance genes in the study sites.

#### 4. Discussion

This work aimed to map the resistance genes of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-resistant (PDR) bacteria to antimicrobials isolated from various pathological products, as well as to monitor the evolution of these bacteria from 2012 to 2025. The geographical distribution of resistance

genes, multidrug-resistant, and extensively resistant bacteria in the different provinces was one of the first of its kind of research activities in Chad, with the introduction of new terms to describe the magnitude of multidrug resistance: MDR, XDR, and PDR. During 24 years (2012-2025) of study, 851 phenotypes of resistant bacteria were identified, including 329 MDR (bacterial isolates remain resistant to at least three or more classes of antibiotics), 407 ESBL (Extended-spectrum beta-lactamases), 114 XDR (bacterial isolates remain sensitive to antibiotics in only one or two classes), and 10 PDR (bacterial isolates remain resistant to all classes of antibiotics tested locally). Among the 10 PDR, there are 3 *Pseudomonas aeruginosa*, 3 *Escherichia coli*, 2 *Proteus* spp., and 2 *Klebsiella pneumoniae*. The prevalence of antimicrobial resistance (AMR) is a growing global threat, already causing more than 700,000 deaths annually and projected to reach 10 million by 2050. Recent WHO reports indicate an increase of more than 40% in resistant infections, with high rates (>30%) for urinary tract infections, and more than 40% of *E. coli* infections and 55% of *K. pneumoniae* infections resistant to first-line antibiotics. Bacteria such as pneumococcus and enterococci are also showing increased resistance, affecting countries previously unaffected and threatening essential treatments. The risk of antibiotic resistance varies from country to country. The WHO estimates that antibiotic resistance is highest in the South-East Asia and Eastern Mediterranean Regions, where one in three reported infections was resistant. In the African Region, one in five infections was resistant. Resistance is also more prevalent and worsens where health systems lack the capacity to diagnose or treat bacterial infections. “Antimicrobial resistance is outpacing the progress of modern medicine and threatens the health of families worldwide,” said Dr. Tedros Adhanom Ghebreyesus, WHO Director-General. “As countries strengthen their antimicrobial resistance surveillance systems, we must use antibiotics responsibly and ensure everyone has access to the right medicines, quality-assured diagnostic tools, and vaccines. Our future also depends on strengthening infection prevention, diagnosis, and treatment systems, as well as developing next-generation antibiotics and rapid molecular tests that can be used at the point of care,” he added [21] [22]. The increase in antimicrobial resistance rates in Chad could be due to increased geographic cross-transmission of drug-resistant drugs. The most probable causes would be self-medication, abusive prescriptions of antimicrobials without laboratory evidence in human and veterinary medicine, the discharge of drugs in garbage heaps around homes and in city gutters, animal and human waste with antibiotic residues in the water environment, and non-compliance with dosages.

Non-duplicated *Mycobacterium leprae* isolates from leprosy patients in three health districts (Abéché, Bebedjia, and N'Djamena) in Chad enabled the identification of the genes (*rpoB*, *floP1*, and *gyrA*). A mutation at codon 456 (Ser-Leu: TCG > TTG) (Ser456 > Leu) of *rpoB*, known to cause rifampicin resistance in relapsed cases, was observed in patients in N'Djamena and was identified with a mutation rate of 1.2%. The frequency of rifampicin resistance (1.2%) was lower

than in Niger (8.3%) [23] but higher than in Senegal and Madagascar (0%) [24]. The *gyrA* sequence also showed a single mutation at codon 91 with an amino acid change from alanine to valine (Figure 3), demonstrating resistance to ofloxacin. The *gyrA* sequence also showed a single mutation at codon 91 with an amino acid change from alanine to valine (GCA > GTA) (Ala91 > Val), demonstrating resistance to ofloxacin. However, the frequency (1.2%) was higher than in African countries and lower than that observed in China (2.9%) for ofloxacin [24] [25].

*Mycobacterium tuberculosis* isolates from tuberculosis patients in hospital and community settings in Chad have allowed the identification of resistance genes, including 13% (*rpoB*) to rifampicin, 4% (*inhA*) to isoniazid, and 8% (*rpoB/KatG* and/or *KatG/inhA*) multidrug-resistant (*mdr*) genes, using the Line Probe Assay technique (Figure 3).

Among the genes detected with mutations associated with antibiotics (Levofloxacin, Amikacin, Kanamycin, and Capreomycin) by the Line Probe Assay technique, 13.6% (*gyrA* and *gyrB*) were due to gyrase enzymes, 7.6% (*eis*), 6.1% (*rrs* and *eis*), and 10.6% were mutations affecting different alleles at the chromosome level of the *Mycobacterium tuberculosis* complex [26].

The mechanism of resistance to levofloxacin was manifested by point mutations occurring in specific regions of two enzymes: DNA gyrase A and DNA gyrase B. Mutations in quinolone resistance determinants (QRDR) of gyrase A and B at the loci of these genes were amplified by PCR and sequenced. The DNA sequences were translated into protein sequences. The sequences of these strains were compared to those of certain strains susceptible to ciprofloxacin to search for amino acid substitutions conferring resistance. Furthermore, similar results have been obtained in Chad, Africa, and worldwide [27]-[31].

Regarding *Vibrio cholerae* O1 strains (Ogawa serotype (98.9%) and Inaba serotype (1.1%)) isolated in Chad from 1971 to 2025, Chad experienced a total of 45 cholera epidemics with 86,961 cases and 6567 deaths, representing a case fatality rate of 7.55%. The strains were confirmed by molecular methods (PCR and PFGE) as carrying the virulence genes encoding the synthesis of cholera toxin (*ctxB* and *ctxA*). All strains were confirmed to be resistant to nalidixic acid (Nal, MIC > 256). The mechanism of nalidixic acid resistance is manifested by point mutations occurring in specific regions of four enzymes: DNA gyrase A, DNA gyrase B, topoisomerase IV C, and topoisomerase IV E. This complementary study focused on mutations in the quinolone resistance determinants (Nal) of the QRD (quinolone resistance determinants) of gyrase A and B. The QRD of the gyrases at the loci of these genes was amplified by PCR and sequenced. The DNA sequences were translated into protein sequences. The sequences of these strains were compared to those of some ciprofloxacin-sensitive strains to search for amino acid substitutions conferring resistance. Several substitutions not present in all ciprofloxacin-sensitive strains were detected, and those involving a marked change in the chemical nature of the amino acid at the substitution position were identified as presumptive resistance-conferring mutations. Cholera toxin virulence genes were de-

tected in 98.61% by PCR and PFGE. Furthermore, Naser *et al.* (2019) and Faruque (1998) showed that plasmid transfer occurs via two types of determinants:

- Type *tetA*, which results in high resistance to oxytetracycline and tetracycline but low resistance to minocycline and doxycycline;
- Type *tetB*, which is expressed by high cross-resistance to all tetracyclines, suggests that our study strains carry the *tetB* determinant [32] [33].

This study detected *Staphylococcus aureus* strains (6.7%) producing penicillinase acquired through modification of PBP (penicillin-binding protein) induced by the *mecA* gene. Six (6) *Staphylococcus aureus* strains resistant to beta-lactams through penicillinase production and to the oxazolidone and sulfonamide families through acquisition of plasmids induced by the *ACC* (6') gene were identified. Resistance of bacterial strains due to membrane protein modification and gene acquisition has also been reported elsewhere [34] [35].

Ten enterococcal strains (*Enterococcus faecalis* (67%) and *Enterococcus faecium* (33%)) isolated in this study exhibited multidrug resistance phenotypes to antibiotics from different families through *vanA* gene induction. Furthermore, studies conducted in England in 1987 and then in the United States from 1990 onward showed that the widespread use of certain antibiotics has contributed to the emergence of 28.5% of resistant enterococcal strains responsible for nosocomial infections in intensive care units. This resistance is of the *vanA* type, conferring resistance to vancomycin and teicoplanin in *Enterococcus faecium*. The *vanA* gene is acquired and transposable to other bacteria such as streptococci and *Staphylococcus aureus*. According to several studies, with regard to such bacteria, especially *Enterococcus faecium*, which have acquired resistance to amoxicillin and glycopeptides, the number of antibiotics likely to be active is very limited, and combinations including aminoglycosides may prove to be poorly or not at all synergistic [36].

Two strains of *Neisseria meningitidis* insensitive to ceftriaxone were isolated in Goundi, Mandoul Province (Figure 3). Although cephalosporins (such as ceftriaxone) remain the standard treatment for invasive *Neisseria meningitidis* infections, strains resistant or with reduced susceptibility to these antibiotics are emerging and being reported worldwide, particularly in Europe and North America, posing a therapeutic challenge. These resistances, primarily to penicillin but also to third-generation cephalosporins, require constant monitoring and antibiograms to guide treatment selection [37].

*Chlamydia trachomatis* was more representative, with a proportion of 25.30%. This result is comparable to that of Sogodogo (2014) in Mali, who found 25.8% of cases of infection, but rather with *Ureaplasma urealyticum* in women suffering from infertility [38]. This difference could be explained by the fact that many women refused to provide a cervical swab. Antibiotic resistance of *Mycoplasma hominis* was determined using the MYCOPLASMA IST-3 kit (BioMérieux, Marcy l'Etoile, France). Resistance rates ranged from 82% to 91% for fluoroquinolones, from 27.27% to 36.36% for macrolides, and reached 36.36% for tetracycline. In

contrast, antibiotic resistance of *Ureaplasma* spp. ranged from 33.33% to 39% for fluoroquinolones, 28% for lincosamides, and reached 39% for tetracycline. The results of this study are consistent with those of previous work conducted in France, China, and Mali [39]-[41].

Finally, 72 fungal agents were identified (51 *Candida albicans*, 3 *Cryptococcus laurentii*, 1 *Candida crusei*, 8 *Candida lipolytica*, 4 *Apergillus niger*, 5 *Apergillus fumigatus*) with varying degrees of resistance to antifungals (azoles (5-fluorocytosine, econazole, clotrimazole, fluconazole, voriconazole) and polyenes (amphotericin B, nystatin)). The fungal strains were susceptible (83.63%) to azole derivatives and resistant (96%) to polyenes. Azole derivatives are more active than polyenes. The activity of fluconazole is limited. We recommend that prescribers prioritize the use of 5-fluorocytosine, econazole, and voriconazole, which are more active and can be considered alternatives to fluconazole. Furthermore, similar studies were reported [42]-[44].

## 5. Conclusion

This study, the first of its kind in Chad, enabled the mapping of resistance genes in emerging multidrug-resistant (MDR), highly resistant (XDR), and pan-resistant (PDR) bacteria to antimicrobials, and the monitoring of healthcare-associated infections from 2012 to 2025. It highlights a major threat posed by the circulation of these bacteria. Controlling the spread of MDR, XDR, and PDR bacteria can only be achieved through cooperation between microbiologists, infection control specialists, clinicians, and the entire healthcare team, as well as all health partners in Chad, Africa, and worldwide.

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## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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