Integrated approach for the detection of bacterial resistance in Mali using chromogenic media

Abstract:

This study, focusing on bloodstream infections and conducted in a rural setting in Mali, aimed to assess bacterial resistance using a two-step diagnostic approach. The first step consisted of preliminary on-site detection of bacteria using manually prepared CHROMagar media, providing a simple, rapid, and cost-effective method. The second step involved phenotypic and genotypic confirmation of isolates in a reference laboratory using advanced techniques such as MALDI-TOF MS, VITEK® 2, and conventional PCR to validate resistance profiles.

Among the 508 blood cultures analyzed, 29.1% (148/508; 95% CI [25–33]) were positive, identifying 16.9% (86/508; 95% CI [13.7–20.2]) resistant strains. Of these, 75.6% (65/86; 95% CI [66.6–84.6]) were Enterobacteriaceae resistant to β-lactams and carbapenems, 13.9% (12/86; 95% CI [6.5–21.3]) corresponded to methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), and 10.5% (9/86; 95% CI [4–16.9]) to other bacterial species.

The most frequently detected resistance genes included CTX-M in 39% (49/126; 95% CI [30.3–47.5]), TEM/SHV in 26% (32/123; 95% CI [18.2–33.8]), genes encoding carbapenemases (KPC, VIM, NDM, OXA-48) in 15% (19/127; 95% CI [8.7–21.3]), and genes encoding other β-lactamases (OXA-1, bla-BEL, bla-ADCb, FOX-1) in 9% (11/122; 95% CI [4–14]). Among *Staphylococcus* and *Enterococcus* isolates, the mecA and vanA/vanB genes were detected in 3% (4/133; 95% CI [0.1–5.9]) and 4% (5/125; 95% CI [0.6–7.4]) of strains, respectively.

CHROMagar media showed a sensitivity of 80% (69/86; 95% CI [71.2–88.8]), a specificity of 85% (73/86; 95% CI [75–91.9]), a positive predictive value of 90% (77/86; 95% CI [82.4–95.1]), and a negative predictive value of 72% (62/86; 95% CI [61.6–80.9]). These findings confirm the potential of this simplified approach to strengthen bacterial resistance surveillance in resource-limited settings, while highlighting the need for reference laboratory validation and protocol optimization to improve its integration into decentralized health systems.

Keywords: CHROMagar media; MRSA; VRE; resistant Enterobacteriaceae; MALDI-TOF MS; VITEK® 2; PCR.

1. Introduction

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi, and parasites evolve over time or no longer respond to medications, making infections more difficult to treat and thereby increasing the risk of disease spread, severe illness, and death [1]. It is essential to obtain information on the current magnitude of the AMR burden, on trends across different parts of the world, and on the main pathogen-drug combinations that contribute to its increase[2].AMR represents a growing threat to global public health, particularly in low- and middle-income countries (LMICs), where limited access to healthcare services and to quality antibiotics exacerbates the situation[2].Each year, approximately 1.27 million deaths are attributed to antimicrobial resistance (AMR) worldwide [2]. In the World Health Organization (WHO) African Region alone, about 250,000 deaths were directly linked to AMR in 2024, highlighting the growing ineffectiveness of conventional treatments against resistant bacterial infections. This trend is further exacerbated by the increasing resistance to last-resort antibiotics, such as carbapenems among Enterobacteriaceae, including *K. pneumoniae*, for which resistance rates rose from less than 1% in 2001 to approximately 15,1% globally among bloodstream infections in 2019, according to published epidemiological estimates [3], [4].

As a result of antimicrobial resistance, antibiotics and other antimicrobial drugs are losing their effectiveness, and infections are becoming increasingly difficult, or even impossible, to treat [1]. One of the major challenges in combating antimicrobial resistance is understanding its true burden, particularly in settings where surveillance is limited and data availability is scarce [2].The spread of AMR is not confined to a few bacterial species but involves a broad range of pathogens. For example, *Staphylococcus aureus*, a bacterium implicated in numerous hospitaland communityacquired infections, exhibits alarming rates of methicillin resistance (methicillin-resistant *Staphylococcus aureus (S. aureus)*: MRSA). According to data from the WHO Global Antimicrobial Resistance Surveillance System (GLASS), the proportion of resistant strains reaches up to 50% in several countries, particularly in Africa, Asia, and Latin America, with a global median rate of 34.7% in bloodstream infections [5], [6].This resistance complicates the treatment of both common and severe infections, such as skin andrespiratory tract infections. Similarly, vancomycin resistance among *enterococci* (VRE), another last-resort antibiotic, affects up to 30% of isolates depending on the region, making the treatment of urinary tract and intra-abdominal infections morechallenging[5]. Furthermore, bacteria belonging to the *Enterobacteriaceae* family, such as *Escherichia coli* (*E. coli*) and *K. pneumoniae*, represent another major concern due to their ability to produce extended-spectrum β-lactamases (ESBLs), which confer resistance to multiple classes of antibiotics, including cephalosporins, with global resistance rates reaching up to 40% [3].

The increasing resistance to carbapenems among *Enterobacteriaceae* adds a further dimension to this global crisis, with resistance rates reaching up to 15% in certain regions. These figures may reflect not only the misuse of antibiotics but also inadequate surveillance and suboptimal management of antimicrobial prescriptions[3].Therapeutic failures associated with bacterial resistance represent a growing threat to global public health, particularly with regard to infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) [7]. These multidrug-resistant strains are especially difficult to treat due to the ineffectiveness of conventional antibiotics, leading to therapeutic failure rates of up to 50% in some cases [7].In Europe, antimicrobial resistance remains a major public health challenge, with concerning resistance rates among *Enterobacteriaceae* reaching up to 30% depending on the country [8],[9]. Resistant bacteria are responsible for approximately 33,000 deaths annually in Europe, underscoring the urgency of a coordinated response to curb the spread of AMR at the continental level [9]. In Asia, E. coli resistance to fluoroquinolones, commonly used to treat urinary tract infections, exceeds 50% in several regions [10].In Africa, antimicrobial resistance represents a growing threat, exacerbated by the uncontrolled use of antibiotics and limitations in healthcare infrastructure that hinder effective surveillance [11].MRSA rates exceed 40% in many regions, largely due to the lack of strict regulatory frameworks [11]. According to an analysis published in The Lancet Global Health, the WHO African Region recorded approximately 1.05 million deaths associated with bacterial AMR in 2019, based on estimates from 47 African countries [12]. In Mali, one study reported that 25% of *Enterobacteriaceae* isolates were ESBL producers, highlighting the magnitude of the problem in the country [13]. Furthermore, although carbapenem resistance is generally less prevalent or less well documented in Africa, another study reported a resistance rate of 5% in Mali, which remains a cause for concern [8].

Conventional bacteriological diagnostic methods are limited in rural settings due to the lack of equipment, the absence of cold-chain facilities for reagent storage, prolonged turnaround times, and difficulties in detecting certain antibiotic resistance mechanisms [14]. These constraints, compounded by insufficiently trained personnel and limited financial resources, delay the management of infections and promote empirical antibiotic use, thereby exacerbating the problem of antimicrobial resistance.In the face of this crisis, a central question emerges: can accessible alternative diagnostic methods be implemented in rural health facilities in LMICs to expand bacteriological diagnostic capacity within local communities? By improving access to early diagnosis and enhancing the surveillance of bacterial resistance in low-resource settings, it would be possible to better target treatments, promote more rational antibiotic use, and curb the spread of antimicrobial resistance. International guidelines on antimicrobial resistance (AMR) surveillance, established by the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (WOAH), are based on the One Health approach and the Global Antimicrobial Resistance and Use Surveillance System (GLASS) to harmonize data collection. These guidelines recommend that countries strengthen their surveillance capacities, particularly across human, animal, and environmental health sectors. In Mali, AMR surveillance is conducted through five sentinel hospital sites, coordinated by the National Institute of Public Health (INSP) and supported by the WHO through the "KOICA" Mali project. According to WHO Africa, Mali 2024, this system enables the collection of data to inform health policies and improve the management of infections [15].

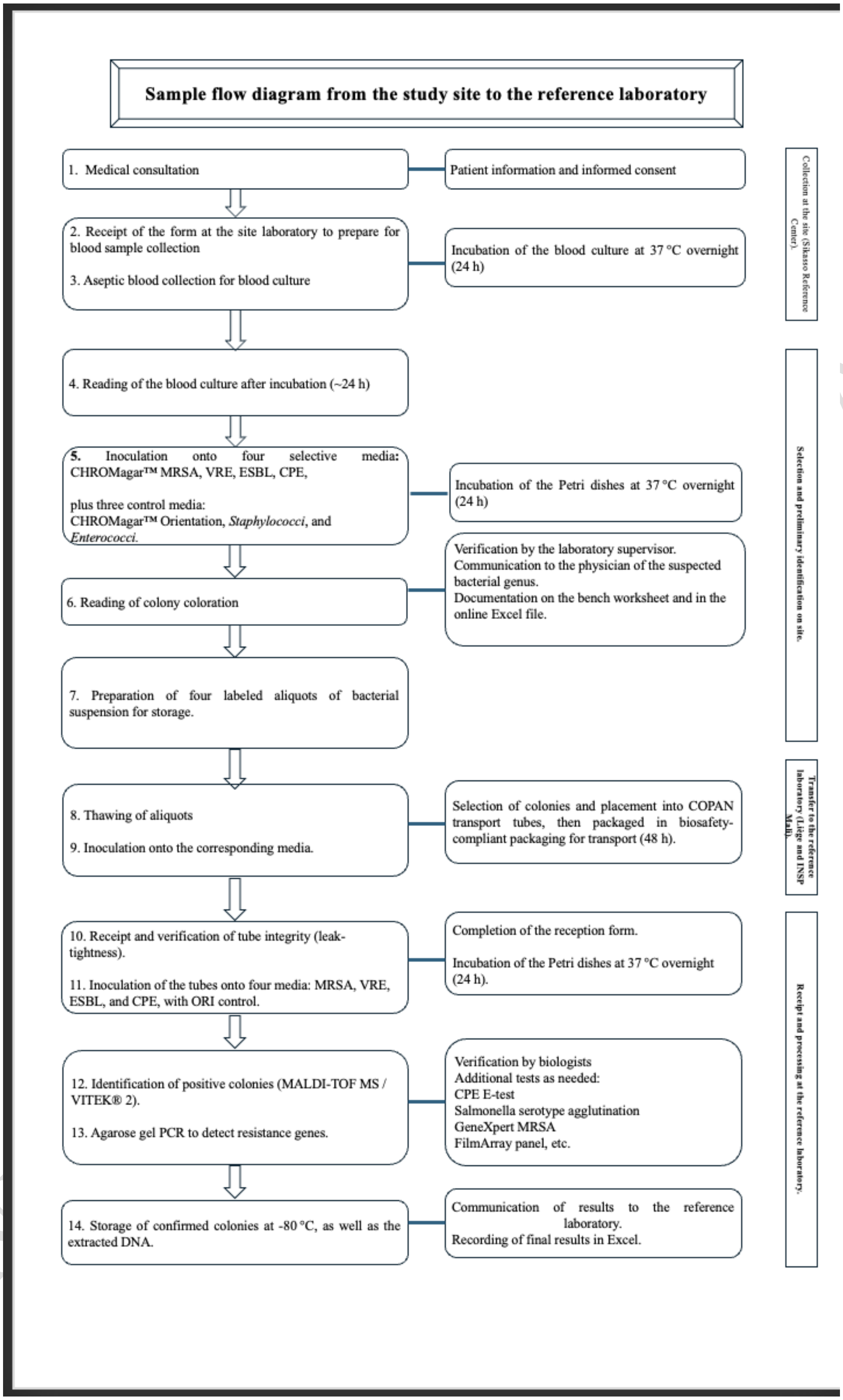
The objective of the present study is to validate the use of a simplified method for the identification of the four main resistant bacterial phenotypes using selective and differential chromogenic media. This method aims to efficiently detectMRSA, VRE, CPE, and ESBL, in order to rapidly communicate results to clinicians and accelerate patient management. These rapid tests will subsequently be compared with confirmatory tests. In parallel, the study will identify the antibiotics to which the isolated strains exhibit resistance, highlighting cases of therapeutic failure and contributing to improved guidance of antibiotic treatment. To assess the accuracy and effectiveness of this approach, results obtained from strains collected in pilot facilities (referral health centers) in Sikasso, Mali, have been analyzed and compared with results generated by the microbiology laboratory of the University of Liege (Belgium), considered as the reference laboratory. This work will contribute to antimicrobial resistance surveillance and to the evaluation of the reliability of the simplified method, with a view to its integration into local health centers. This approach aims to improve the management of resistant infections by prescribers, while ensuring homogeneous and standardized data collection on bacterial resistance in resource-limited settings.

2. Materials and Methods

2.1. Study Design

Sample collection was conducted between August 2020 and April 2024 in several healthcare facilities in Sikasso, Mali, according to the following sample flow diagram:

Figure 1: Sample flow diagram from the study sites to the reference laboratory



2.2. Health facilities

The Sikasso Reference Health Center (CSRef), Mali, served as the focal point of the study. Several healthcare facilities in the region were also involved as satellite centers, including the

Niena Reference Health Center, Avenir Clinic of Sikasso, Bassaran Medical Clinic of Sikasso, and the Mamassoni Community Health Center (CSCom) of Sikasso.

2.3. Inclusion criteria

All patients admitted to the reference health center or to the satellite centers were included in the study if they met at least one of the following criteria:

- Persistent unexplained fever (>10–14 days despite antibiotic treatment) or intermittent fever (temperature ≥ 38.5 °C), or hypothermia (< 36 °C)
- Suspected systemic infection (sepsis or septic shock)
- Localized infection with a risk of dissemination
- Signs of septic thrombophlebitis or suspected catheter-related infection
- Suspected medical device–associated infection
- Presence of biological markers of organ dysfunction [16], [17],[18].

2.4. On-site collection of resistant bacterial strains

2.4.1. Blood cultures

In this study, two types of blood culture bottles were used. The Signal™ system (Thermo Scientific™, France), suitable for both adults and children and requiring minimal equipment [19], was initially employed and was particularly adapted to laboratories of peripheral reference medical facilities (PRMFs). However, toward the end of the study, aerobic BacT/Alert bottles (bioMérieux, France) were used instead of the latter due to logistical constraints, as the supplier was unable to ensure delivery of the Signal™ system to the study area in Mali. It should be noted that the first shipment of the Signal™ system was delivered to Liege and subsequently transported to the study site by the study supervisor. In contrast, BacT/Alert bottles were readily available and could be delivered locally. This change was implemented after verifying the feasibility of visually reading the color change of the indicator pellet.

Each bottle was inoculated with 10 mL of adult blood or 2–5 mL of pediatric blood collected in accordance with World Health Organization (WHO) recommendations. The bottles were incubated at 37 °C for up to five days (TITANOX incubator, Italy). A positive result was detected by a pressure change (Signal™) or a color change (BacT/Alert). This is followed by inoculation onto culture media.

2.4.2. Subculture media used on site and management of cultured strains

After incubation, the broth from positive blood culture bottles was subcultured onto various media prepared in accordance with the manufacturer's recommendations (CHROMagar™).Each sample was inoculated onto two groups of culture media previously validated in CHU Liege:

Group 1: CHROMagar™ ESBL, CHROMagar™ CPE, CHROMagar™ MRSA, and CHROMagar™ VRE.

Group 2: CHROMagar™ Orientation, CHROMagar™ *Staphylococcus*, and CHROMagar™ *Enterococcus*.

The chromogenic media used in this study were previously validated at the CHU Liege laboratory in accordance with internal protocols and the manufacturer's recommendations[20].Plates were incubated aerobically at 37 °C for 24 h. Results were recorded based on colony coloration [21].The strains cultured on these selective media were stored in 5-mL transparent screw-cap vials with black caps (Paracelsus Versand, Germany) containing 2 mL of sterile glycerol. Each strain was stored in triplicate. One of the tubes was subsequently subcultured and transferred using an individually packaged sterile plastic-shaft swab with a rayon tip into a transport tube containing Amies gel medium without charcoal (Amies Agar Gel w/o charcoal), designed for the transport of aerobic and anaerobic bacteria (COPAN, USA). The prepared samples were shipped to the Clinical Microbiology Laboratory (CML) of the University of Liege (ULg), Belgium, for confirmatory analysis. Transport was performed using certified transport kits (Tennant Packaging Corporation [TPC], Atlanta, USA) under ambient conditions by air within 48 h. From September 2024 onward, a second tube was sent to the National Institute of Public Health of Mali for confirmatory analysis to ensure the sustainability of the study. A third tube was retained and stored on site as a backup.

Table 1:Characteristics of colonies of resistant bacteria on CHROMagar media according to the manufacturer

Culture medium	Bacterial colony characteristics
CHROMagar™ ESBL	*Escherichia coli*: dark pink to reddish colonies
	KEC (*Klebsiella, Enterobacter, Citrobacter*): metallic blue colonies with or without a reddish halo
	Proteus spp.: brown halo
	Acinetobacter spp.: cream-colored colonies
	Pseudomonas spp.: translucent colonies, with or without natural pigmentation (cream to green)
	Stenotrophomonas spp.: colorless colonies
CHROMagar™ VRE	*Enterococcus faecium* and *Enterococcus faecalis*: pink to mauve colonies
	Enterococcus gallinarum and *Enterococcus casseliflavus*: blue colonies or inhibited growth
CHROMagar™ MRSA	MRSA (methicillin-resistant *Staphylococcus aureus*): pink to mauve colonies
	MSSA (methicillin-susceptible *Staphylococcus aureus*) and other bacteria: inhibited growth
CHROMagar™ mSuperCARBA	*Escherichia coli*: dark pink to reddish colonies
	Coliforms: metallic blue colonies
	Pseudomonas spp.: translucent colonies, with or without natural pigmentation (cream to green)
	Acinetobacter spp.: cream-colored colonies
	Other Gram-negative CPE: colorless colonies with natural pigmentation

2.4.3. Contamination control of culture media

To monitor contamination over time, for each batch of prepared culture media, one control plate was reserved for quality control procedures: a sterility control (absence of growth) and a positive control, performed using a reference strain to confirm the quality and performance of the medium [22].

2.5. Analysis of antibiotic prescription frequency in rural settings: a descriptive study of prescribed agents

Data were extracted from prescription registers and archived medical prescriptions at the reference health center during the study period. Each prescription was analyzed to identify the active substance, associated commercial formulations, and the cumulative prescription frequency, expressed as a percentage.Antibiotics were classified according to their active ingredient, grouping together the different pharmaceutical specialties available locally. This methodology allowed the characterization of prevailing therapeutic practices, highlighting the most frequently prescribed antibiotics, often used empirically in the absence of microbiological confirmation. Prescription data were subsequently compared with antimicrobial susceptibility results, distinguishing susceptible, intermediate, or resistant profiles for each evaluated agent.

2.6. Confirmation at the reference laboratory

Upon receipt of the samples at the CML Liege laboratory, tube integrity was verified and documented. Colonies were subsequently subcultured within 24 hours of receipt, following the same protocol applied at the study site in Mali.

2.6.1. MALDI-TOF MS identification

The MALDI Biotyper (software version 3.0, Bruker, Germany) was used at the laboratory of CHU Liege. Microorganisms were spotted onto a target plate and overlaid with 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix [23], [24].Scores ≥ 1.7 were considered acceptable for colony identification when they were consistent with previous identification results.

2.6.2. Antimicrobial susceptibility testing

2.6.2.1. Manual antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method in accordance with current standards. For Gram-negative bacteria, testing was carried out on Mueller–Hinton agar. The antibiotics tested included meropenem (MEM), cefepime (FEP), ceftazidime (CAZ), amoxicillin–clavulanic acid (AMC), cefotaxime (CTX), piperacillin–tazobactam (TZP), and ciprofloxacin (CIP). As regards Gram-positive bacteria, the antibiogram was performed on bioMérieux Mueller-Hinton (MH) agar. The antibiotic disks tested included teicoplanin (TEC), vancomycin (VA), cefoxitin (FOX), and mupirocin (MUP).Inhibition zone diameters were measured, and results were interpreted according to standardized susceptibility criteria (EUCAST version 10.0, 2020) [25],[26].

2.6.2.2.VITEK® 2 automated susceptibility testing

Each bacterial isolate was suspended in VITEK® 2 Suspension Medium (bioMérieux, France), and the suspension turbidity was adjusted to 0,5 McFarland [24].The standardized suspension was then used to inoculate antimicrobial susceptibility testing (AST) cards on the VITEK® 2 system (bioMérieux, France). AST cards 652 and 655 were used for Gram-positive bacteria, while cards 366 and 367 were used for Gram-negative bacteria, in accordance with the manufacturer's instructions. Identification results obtained by VITEK® MS were integrated into the VITEK® 2 system via MYLA to minimize analytical errors, using only the recommended criteria (EUCAST Standard Operating Procedure (SOP) version 10.2, 2021). Minimum inhibitory concentrations (MICs) were interpreted into clinical categories (susceptible, susceptible with increased exposure, or resistant) in accordance with EUCAST recommendations (EUCAST version 10.0, 2020; EUCAST version 11.0, 2021).

2.6.3. Polymerase chain reaction (PCR)

2.6.3.1.DNA extraction

Bacterial colonies suspended in sterile saline and adjusted to 0.5 McFarland were incubated for 20 minutes at 56 °C in AL buffer (Qiagen, Germany) with proteinase K (Qiagen, Germany), followed by purification using magnetic beads. Genomic DNA was extracted using the Maxwell® 48 instrument (Promega, USA) with the Maxwell® Cell DNA kit, following the manufacturer's instructions[27]. Extracted DNA was eluted in 60 µL of elution buffer and stored at −20 °C for subsequent analyses.

2.6.3.2.Detection of antimicrobial resistance genes

To characterize the molecular mechanisms of antimicrobial resistance, a set of oligonucleotide primer pairs was used for the specific amplification of targeted bacterial genes (Table 2). With regard to β-lactamases [28],[29], the genes investigated included bla_BEL, bla_ADCb, OXA-1, multiple CTX-M groups (including groups 9, 1, and 2), multiple TEM groups (including TEM-1 and TEM-2), and SHV-1. The characterisation of carbapenemases focused on the OXA-48, VIM, and NDM genes, in accordance with established recommendations [28], [29]. Methicillin resistance in staphylococci was detected by identifying the mecA gene, and the presence of the FOX-1 gene was also assessed. Regarding glycopeptide resistance, the vanA, vanB, and vanC genes were targeted[30],[31],[32],[33].Finally, the biofilm-forming capacity of the isolates was explored through amplification of the icaB and icaD genes[34].Table 2, which list the different primers used, is provided in the appendix. These sequences were selected based on the scientific literature for their specificity and efficiency in amplifying target regions by PCR, enabling reliable identification of the most common resistance genes in multidrug-resistant bacterial strains.

2.6.3.3.DNA amplification

Amplification of the extracted DNA was performed using a VeritiPro™ 96-Well Thermal Cycler (Thermo Fisher Scientific, USA). The polymerase chain reaction (PCR) mixture was prepared using 2.0 µL of template DNA, to which the specific forward and reverse primers (0.5 µM each; Eurogentec, Belgium), Taq DNA polymerase (0.2 U; Qiagen, Germany), 10× reaction buffer (2.0 µL), dNTPs (0.2 µL), $MgCl_2$ (2.0 µL), and sterile water (12.0 µL) were added[28],[31], [32], [33].

2.6.3.4.Thermocycler conditions

Thermocycler programs were adapted according to the bacterial group (Table 3). Gram-negative and Gram-positive bacteria were subjected to specific conditions of denaturation, annealing, extension, and cycle numbers, in accordance with published protocols [28],[35], [32], [34].

Table 3: Thermocycler program according to bacterial type

Step	**Gram-negative bacteria**	**Gram-positive bacteria**
Initial denaturation	94 °C for 15 minutes (min)	95 °C for 15 min
Number of cycles	40 cycles	44 cycles
Denaturation (per cycle)	94 °C for 30 seconds (s)	94 °C for 40 s
Annealing	57 °C for 90 s	60 °C for 40 s
Extension	72 °C for 90 s	72 °C for 60 s
Final extension	72 °C for 10 min	72 °C for 5 min
Hold	4 °C	4 °C

2.6.3.5. DNA quantification and analysis

DNA was quantified using the Qubit™ Flex High Sensitivity Fluorometer (Thermo Fisher Scientific, USA) to ensure an adequate concentration prior to electrophoresis, with a minimum required threshold set at 10 ng/µL [36], [37].

2.7.Statistical analysis

The collected data were entered into an Excel spreadsheet for statistical analysis. Qualitative and quantitative variables were summarized using descriptive statistics [21].A 2 × 2 contingency table was constructed to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of CHROMagar compared with the reference test for the analyzed samples [21].

2.8.Cost analysis

The cost of analysis was estimated using an Excel spreadsheet, based on the costs of consumables, labor, equipment, time (including instrument usage, analytical procedures, and culture media preparation), energy, and transportation.

3. Results

Table 4 presents the demographic and clinical characteristics, as well as blood culture results, of the patients included in the study, with 95% confidence intervals for each variable.

Table 4:Number and proportion observed among variables with available data (95% CI)

Variable	N reported	N Observed (95% CI)
Positive blood cultures	508	148 (29%, CI 25–33)
Local residents	352	330 (94%, CI 91–96)
Mean age ± SE (years)	291	30 (CI 27–33)
Outpatients	316	270 (85%, CI 81–89)
Negative malaria test	316	227 (72%, 95% CI 67–76)
Female	367	202 (55%, 95% CI 50–60)
Male	367	165 (45%, 95% CI 40–50)

n: number; CI: confidence interval

Out of 6,638 prepared culture plates, 109 were contaminated, corresponding to a contamination rate of 1.64%. The study, conducted on 508 blood cultures between August 2020 and April 2024, was carried out in four phases: implementation of the system (7 months), initiation of sample collection (12 months), data collection and expansion to additional healthcare facilities (25 months), and sustainability through the transfer of samples to the National Institute of Public Health of Mali (INSP Mali).

Table 5: Distribution of Bacterial Isolates (n = 112)

Bacterial isolates	**Number of isolates (n)**	**Frequency (%)**	**95% CI**
Escherichia coli	49	43.8%	[34.6 – 52.9]
***Enterobacter* spp**	20	17.9%	[10.8 – 25.0]
Klebsiella pneumoniae	13	11.6%	[5.7 – 17.5]
Staphylococcus aureus	17	15.5%	[8.5 – 21.8]
***Enterococcus* spp**	4	3.6%	[0.1 – 7.0]
Pseudomonas aeruginosa	6	5.4%	[1.2 – 9.5]
***Salmonella* spp**	2	1.8%	[0 – 4.2]
Acinetobacter baumannii	1	0.9%	[0 – 2.6]
Other bacteria *(Pseudomonas* spp + *Acinetobacter* spp*)*	7	6.3%	[1.8 – 10.8]
Total	112	100%	—

Table 6 presents the contamination rates of manually prepared culture media between July 2021 and April 2024. The overall contamination rate was low, at 1.64% (109/6,638; 95% CI [1.36–1.97]). CHROMagar™ Orientation (ORI) and CHROMagar™ MRSA media showed the highest contamination rates.

Table 6: Contamination rates of manually prepared CHROMagar™ culture media at the Sikasso Reference Health Center (Mali) between July 2021 and April 2024.

Culture medium	**CPE**	**ESBL**	**VRE**	**MRSA**	**ORI**	**Total**
CHROMagar plates	1,307	1,303	1,479	1,296	1,253	6,638
Contaminated plates (N)	20	22	20	22	25	109
Contamination rate (%)	1.53	1.69	1.35	1.70	1.99	1.64
95% CI (Wilson)	[0.94–2.36]	[1.10–2.56]	[0.88–2.09]	[1.12–2.59]	[1.35–2.94]	[1.36–1.97]

3.1. Resistance profile

3.1.1. Antimicrobial susceptibility testing

The resistance profile shows a very high level of resistance to amoxicillin/clavulanic acid at 94% (32/34; 95% CI [80.9–98.4]), to cefepime and cefotaxime at 91% (31/34; 95% CI [77.0–97.0]), as well as to ceftazidime at 88% (30/34; 95% CI [73.4–95.3]). In contrast, meropenem remains largely active, with only 15% resistance (5/34; 95% CI [6.4–30.1]) (Figure 1).

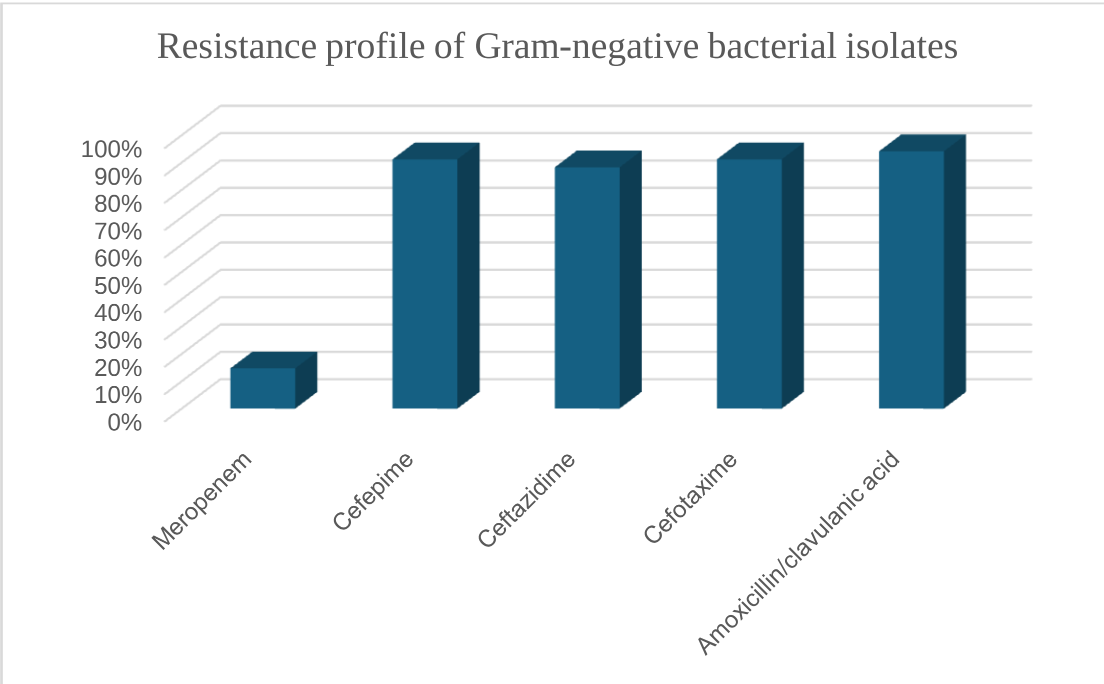


Figure 1:Resistance profile observed among Gram-negative isolates

ESBL: Among *E. coli* isolates, the proportion of ESBL-producing strains was 37% (15/41; 95% CI [23.6–51.9]), whereas non–ESBL-producing strains accounted for 63.4% (26/41; 95% CI [48.1–76.4]). Similarly, *K. pneumoniae* showed a comparable proportion of ESBL producers at 36% (4/11; 95% CI [15.2–64.6]), compared with 63.6% (7/11; 95% CI [35.4–84.8]) of non–ESBL-producing strains. In contrast, *K. aerogenes* exhibited an opposite trend, with a higher proportion of ESBL-producing strains at 55% (6/11; 95% CI [28.0–78.7]) than non–ESBL-producing strains at 45.5% (5/11; 95% CI [21.3–72.0]). Finally, no *Salmonella* or *Pseudomonas* isolates produced ESBLs.

Gram-positive bacteria: Among *S. aureus* isolates, resistance to cefoxitin and mupirocin was observed in 34.8% of cases (8/23; 95% CI [18.8–55.1]). In addition, *Enterococcus* spp. showed resistance to teicoplanin and vancomycin in 42.9% of isolates (3/7; 95% CI [15.8–75.0]).

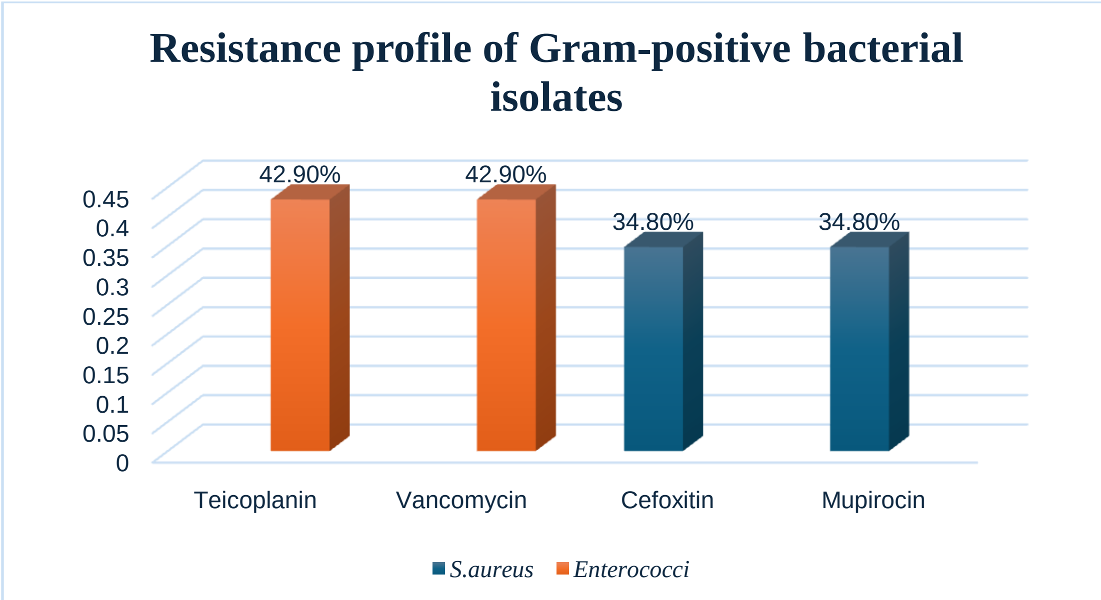


Figure 2. Antibiotic resistance profile observed among Gram-positive isolates

3.1.2. VITEK 2

For *E. coli*, the highest resistance rates were observed for ampicillin, at 95% (41/43; 95% CI [83.5–98.6]), amoxicillin–clavulanic acid, at 92% (33/36; 95% CI [78.6–97.6]), and trimethoprim–sulfamethoxazole, at 90% (46/51; 95% CI [78.6–95.8]). In addition, high levels of resistance were observed to second- and third-generation cephalosporins, notably cefuroxime at 91% (39/43; 95% CI [77.0–97.0]) and cefotaxime at 82% (36/44; 95% CI [67.1–90.9]).In contrast, carbapenems such as meropenem and ertapenem showed preserved activity, with low resistance rates of 6% (3/52; 95% CI [2.0–16.4]) and 19% (8/43; 95% CI [9.8–33.9]), respectively. Finally, a moderate level of resistance was observed for gentamicin at 35% (18/52; 95% CI [23.0–49.5]), while a high level of resistance was observed for ciprofloxacin at 67% (35/52; 95% CI [52.9–78.8]) (Figure 3).

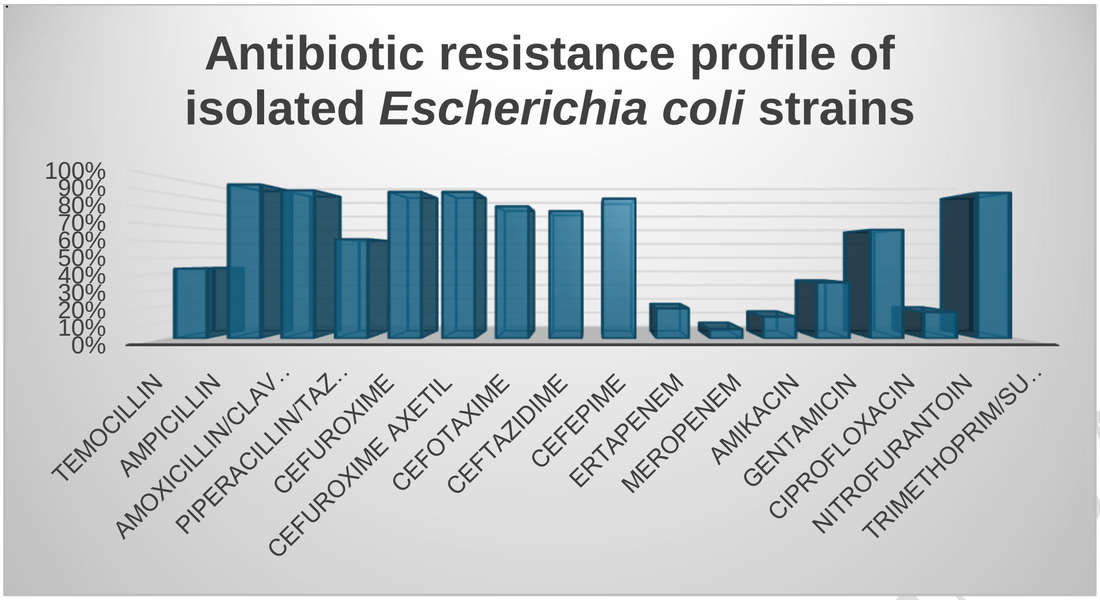


Figure 3: Antibiotic resistance profile of *Escherichia coli* isolates

The isolated *K. pneumoniae* strains exhibited systematic resistance to ampicillin, at 100% (6/6; 95% CI [61–100]), in accordance with the intrinsic resistance profile of this species. Moderate resistance was observed for other penicillins combined with β-lactamase inhibitors, with resistance rates of 50% for amoxicillin–clavulanic acid (2/4; 95% CI [15–85]), compared with only 15% resistance to piperacillin–tazobactam (2/13; 95% CI [4–43]). Regarding second- to fourth-generation cephalosporins, resistance rates were homogeneous and high: 50% (3/6; 95% CI [18–82]) for cefuroxime (and its axetil form), 46% (6/13; 95% CI [23–71]) for ceftazidime and cefepime, as well as for cefotaxime. Carbapenems remained largely effective, with low resistance rates to ertapenem at 17% (1/6; 95% CI [3–56]), meropenem at 8% (1/13; 95% CI [1–36]), and imipenem at 17% (1/6; 95% CI [3–56]).Among aminoglycosides, amikacin retained good activity, with a resistance rate of 15% (2/13; 95% CI [4–43]), in contrast to gentamicin at 54% (7/13; 95% CI [29–77]). Fluoroquinolones such as ciprofloxacin and levofloxacin showed intermediate resistance levels, at 46% (6/13; 95% CI [23–71]) and 43% (3/7; 95% CI [16–75]), respectively.High resistance was observed to trimethoprim–sulfamethoxazole, at 64% (7/11; 95% CI [35–85]), whereas nitrofurantoin remained largely active, with resistance rates of 17% (1/6; 95% CI [3–56])(Figure 4).

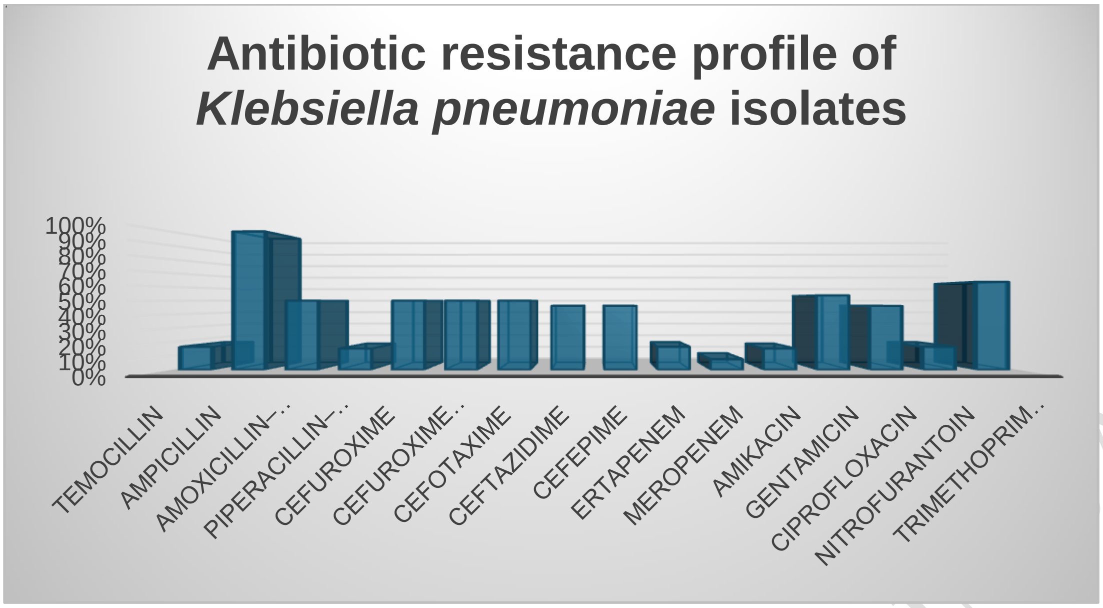


Figure 4. Antibiotic resistance profile of *Klebsiella pneumoniae*

The isolated *K. aerogenes* strains exhibited a high level of resistance to β-lactam antibiotics, with a rate of 71% (5/7; 95% CI [36–92]), particularly to ceftazidime, and aztreonam. Moderate resistance rates were observed for piperacillin/tazobactam, cefepime, and trimethoprim/sulfamethoxazole, each at 43% (3/7; 95% CI [16–75]).Carbapenems remained largely effective, with only 14% resistance (1/7; 95% CI [3–51]) to both meropenem and imipenem. Among aminoglycosides, amikacin showed partialactivity, with resistance rates of 29% (2/7; 95% CI [8–64]), whereas gentamicin retained better efficacy, with a resistance rate of 14% (1/7; 95% CI [3–51]).Fluoroquinolones showed moderate resistance, represented by ciprofloxacin at 43% (3/7; 95% CI [16–75]) (Figure 5).

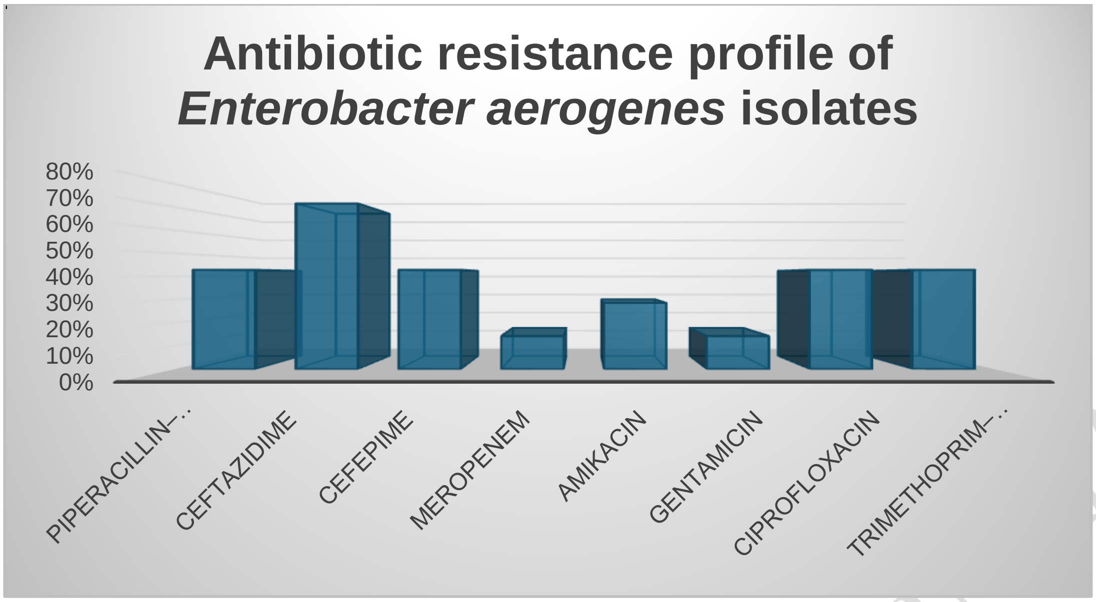


Figure 5. Antibiotic resistance profile of *K. aerogenes*

The isolated *Enterobacter cloacae* strains showed a low rate of resistance to penicillins, with or without β-lactamase inhibitors. Resistance was observed in only 6% of isolates (1/18; 95% CI [1–27]) for ticarcillin and ticarcillin/clavulanic acid, and in 11% (2/18; 95% CI [3–32]) for piperacillin/tazobactam.

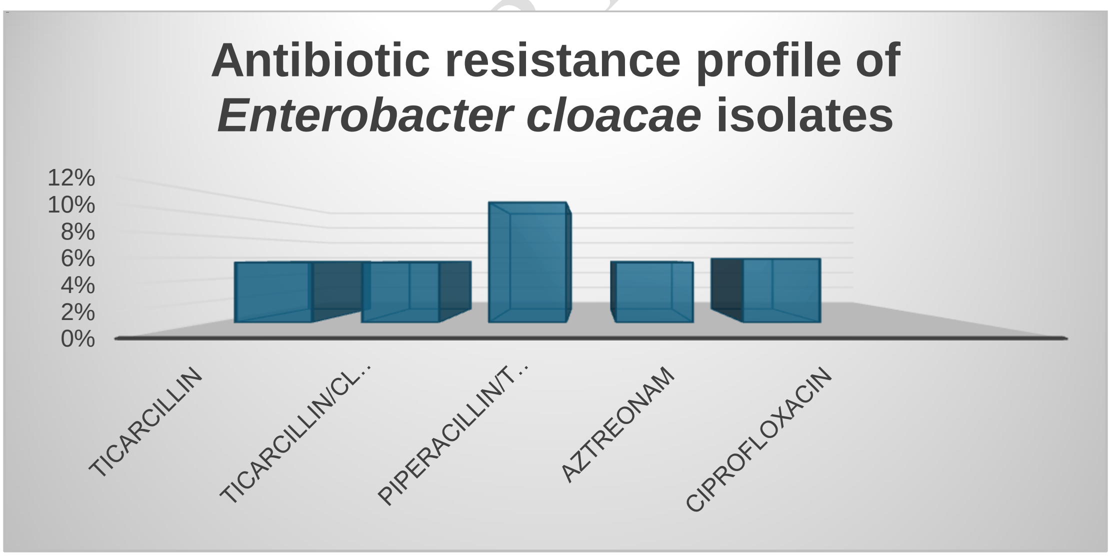


Figure 6. Antibiotic resistance profile of *Enterobacter cloacae*

The isolated *Salmonella enteritidis* strains exhibited a high resistance profile to several classes of antibiotics. Among penicillin's, resistance was observed to ampicillin in 100% of isolates (2/2; 95% CI [34.2–100]). Regarding aminoglycosides, resistance to amikacin was observed in 25% of isolates (1/4; 95% CI [5.2–69.8]). For fluoroquinolones, resistance to ciprofloxacin was detected in 50% of isolates (2/4; 95% CI [15.0–84.8]).Finally, complete resistance to trimethoprim/sulfamethoxazole was observed, with a rate of 100% (3/3; 95% CI [44–100]) (Figure 7).

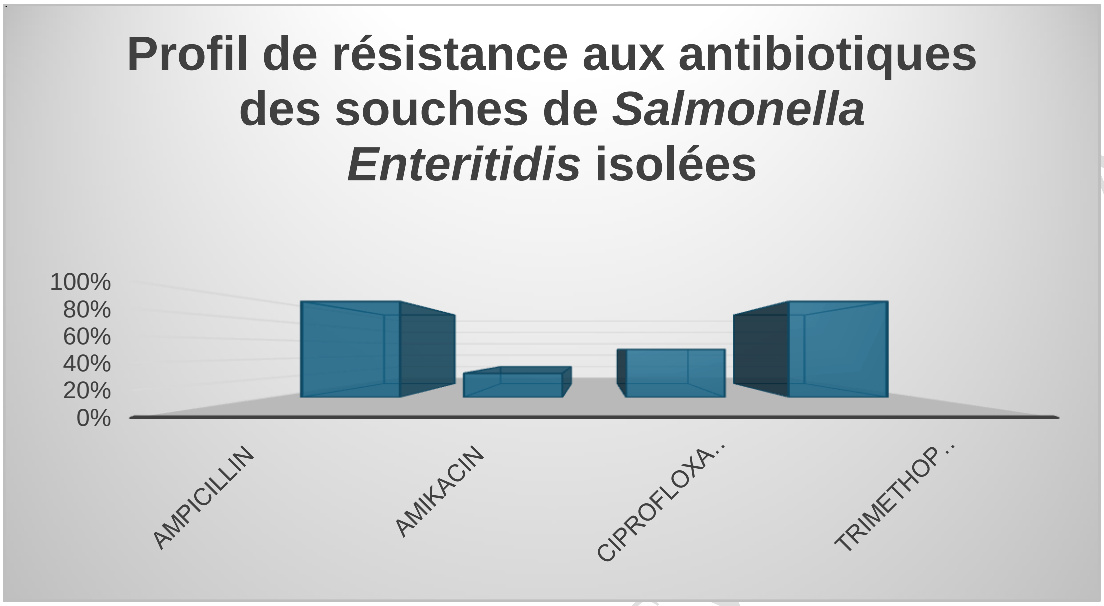


Figure 7. Antibiotic resistance profile of *Salmonella enteritidis*

The isolated *Pseudomonas aeruginosa* strains exhibited high resistance to several β-lactam antibiotics, particularly aztreonam at 75% (6/8; 95% CI [40.93–92.85]), ceftazidime at 57% (4/7; 95% CI [25.05–84.18]), and ticarcillin/clavulanic acid at 56% (5/9; 95% CI [26.67–81.12]). Resistance to piperacillin/tazobactam (38%; 3/8; 95% CI [13.68–69.43]) and cefepime (43%; 3/7; 95% CI [15.82–74.95]) remained moderate. Carbapenems, notably meropenem (11%; 1/9; 95% CI [1.99–43.50]) and imipenem (14%; 1/7; 95% CI [2.57–51.31]), retained good activity. With regard to fluoroquinolones, ciprofloxacin showed moderate resistance at 44% (4/9; 95% CI [18.88–73.33]), whereas levofloxacin exhibited a lower resistance rate of 22% (2/9; 95% CI [6.32–54.74]).Among aminoglycosides, tobramycin demonstrated a resistance rate of 33% (2/9; 95% CI [12.06–64.58]), reflecting partial efficacy (Figure 8).

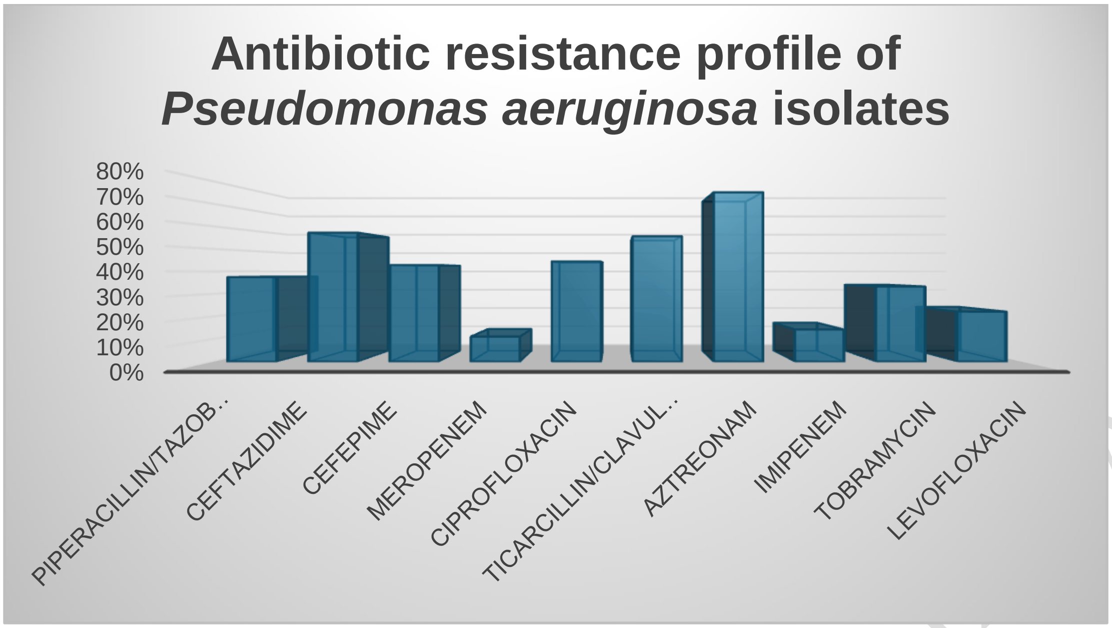


Figure 8. Antibiotic resistance profile of *Pseudomonas aeruginosa*.

The isolated *S. aureus* strains exhibited variable resistance patterns across different antibiotic classes. Among β-lactams, resistance to oxacillin was observed in 29% of isolates (4/14; 95% CI [11.7–54.6]). Aminoglycosides retained good activity, with resistance rates of 7% for gentamicin (1/14; 95% CI [1.3–31.5]) and 14% for kanamycin and tobramycin (2/14; 95% CI [4.0–39.9]). In contrast, resistance to macrolide–lincosamide–streptogramin (MLS) antibiotics was notable, with resistance rates of 47% for erythromycin (7/15; 95% CI [24.8–69.9]) and 60% for clindamycin (9/15; 95% CI [35.7–80.2]). Regarding tetracyclines, resistance was high to tetracycline (73%; 11/15; 95% CI [48.0–89.1]) but lower to minocycline (14%; 2/14; 95% CI [4.0–39.9]). Finally, resistance to trimethoprim/sulfamethoxazole was observed in 47% of isolates (7/15; 95% CI [24.8–69.9]), whereas good susceptibility was noted for fusidic acid (64%; 5/14; 95% CI [16.3–61.2]) and rifampicin (71%; 4/14; 95% CI [11.7–54.6]), highlighting substantial variability in resistance profiles (Figure 9).

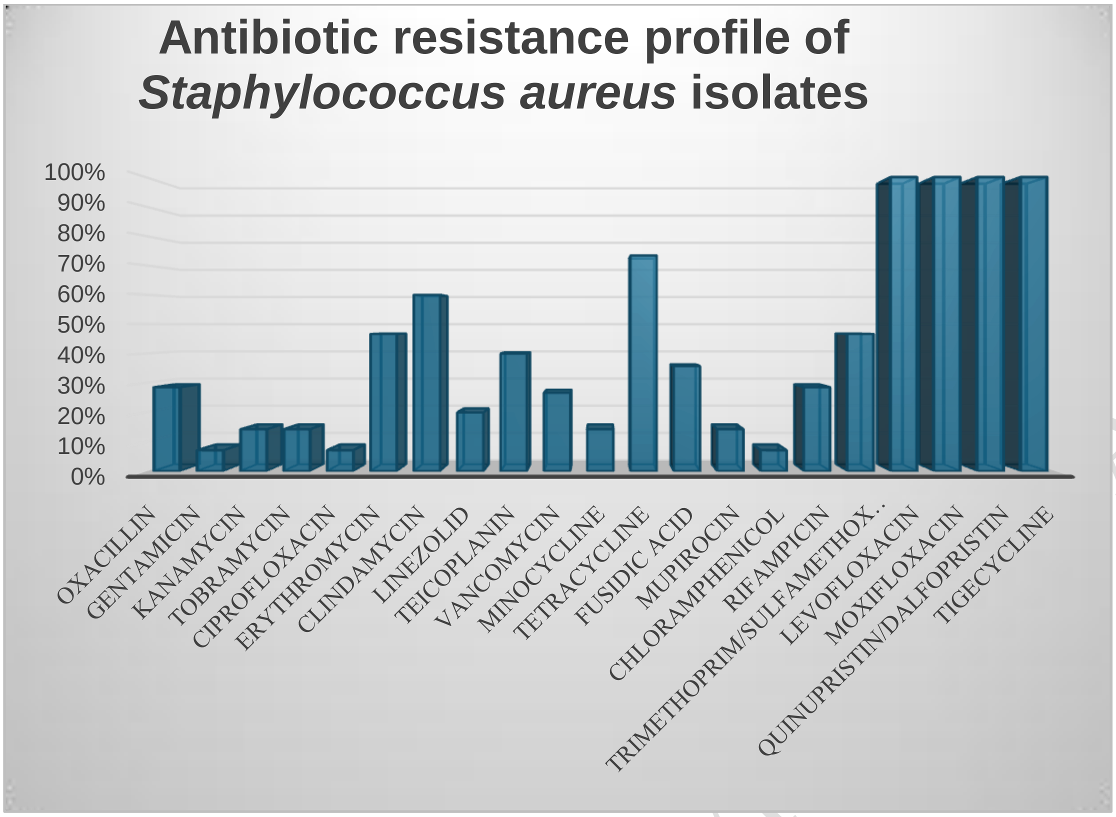


Figure 9. Antibiotic resistance profile of *Staphylococcus aureus.*

The *Enterococcus* spp. strains obtained (two *Enterococcus casseliflavus* and two *Enterococcus faecalis*) exhibited marked multidrug resistance across several antibiotic classes. An intrinsic resistance of 100% (4/4; 95% CI [51–100]) to macrolide–lincosamide antibiotics, particularly clindamycin, was observed. Regarding glycopeptides, substantial resistance was also detected: 50% of isolates (2/4; 95% CI [15–85]) were resistant to both vancomycin and teicoplanin, suggesting the presence of vancomycin-resistant enterococci (VRE). Finally, tigecycline, a glycylcycline antibiotic, showed partial activity, with a resistance rate of 67% (1/3; 95% CI [6.2–79.2]) (Figure 10).

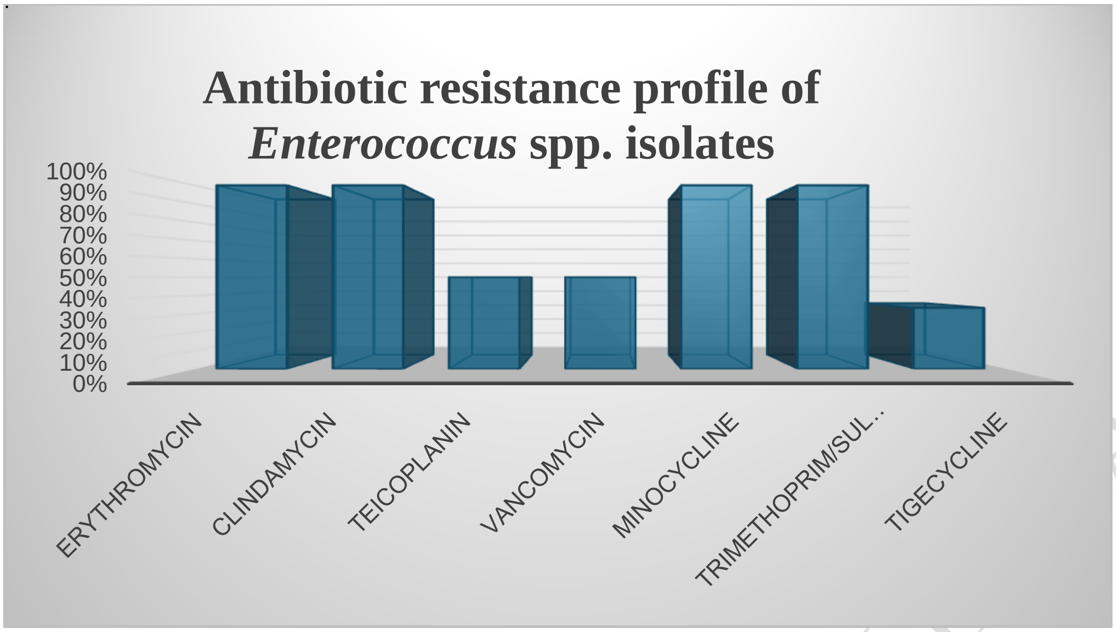


Figure 10. Antibiotic resistance profile of *Enterococcus* spp.

3.2. Resistance profile of the most frequently prescribed antibiotics

The analysis of antibiotics prescribed by clinicians in Mali, based on data collected from medical prescriptions, reveals a clear predominance of cephalosporins and, to a lesser extent, fluoroquinolones, reflecting a substantial reliance on broad-spectrum antibiotics in clinical practice. Ceftriaxone (16%) and cefixime (15%), both third-generation cephalosporins, were among the most frequently prescribed agents, suggesting predominantly empirical use in the management of community-acquired and hospital infections. Azithromycin (18%), a macrolide commonly indicated for respiratory tract infections and certain atypical bacterial infections, emerged as the most frequently prescribed antibiotic overall. Other commonly prescribed antibiotics included cotrimoxazole (12%) and amoxicillin/clavulanic acid (11%), both widely used as first-line treatments, as well as ceftazidime (7%), ciprofloxacin (5%), and levofloxacin (4%), further highlighting the significant role of fluoroquinolones. Less frequently prescribed antibiotics included cefuroxime (4%), ceftriaxone combined with sulbactam (4%), gentamicin (2%), and clarithromycin (2%), likely reflecting more specific indications or more targeted use in particular clinical contexts. Susceptibility testing results were available for only a subset of prescribed antibiotics. High resistance rates were observed for ceftriaxone (84%), the ceftriaxone–sulbactam combination (84%), amoxicillin/clavulanic acid (80%), and cotrimoxazole (66%). Fluoroquinolones also showed substantial resistance, with 51% resistance to ciprofloxacin and 37% to levofloxacin. Ceftazidime exhibited a resistance rate of 57%. In contrast, gentamicin, although infrequently prescribed (2%), showed a high susceptibility rate of 73%. No susceptibility data were available for clarithromycin, cefoperazone, cefixime, or azithromycin.

Among the most frequently prescribed antibiotics, several exhibited high resistance rates, notably ampicillin at 98.45% (45/46; 95% CI [88.7–99.6]), amoxicillin/clavulanic acid at 70.83% (32/45; 95% CI [56.6–82.3]), and cefuroxime at 70.35% (38/54; 95% CI [57.2–80.9]).

In contrast, some less frequently prescribed antibiotics retained better activity, including teicoplanin with a resistance rate of 45% (8/19; 95% CI [19.9–64.3]), gentamicin at 73% (50/68; 95% CI [63.0–84.0]), and amikacin at 89% (80/91; 95% CI [81.2–94.6]) (Figure 11).

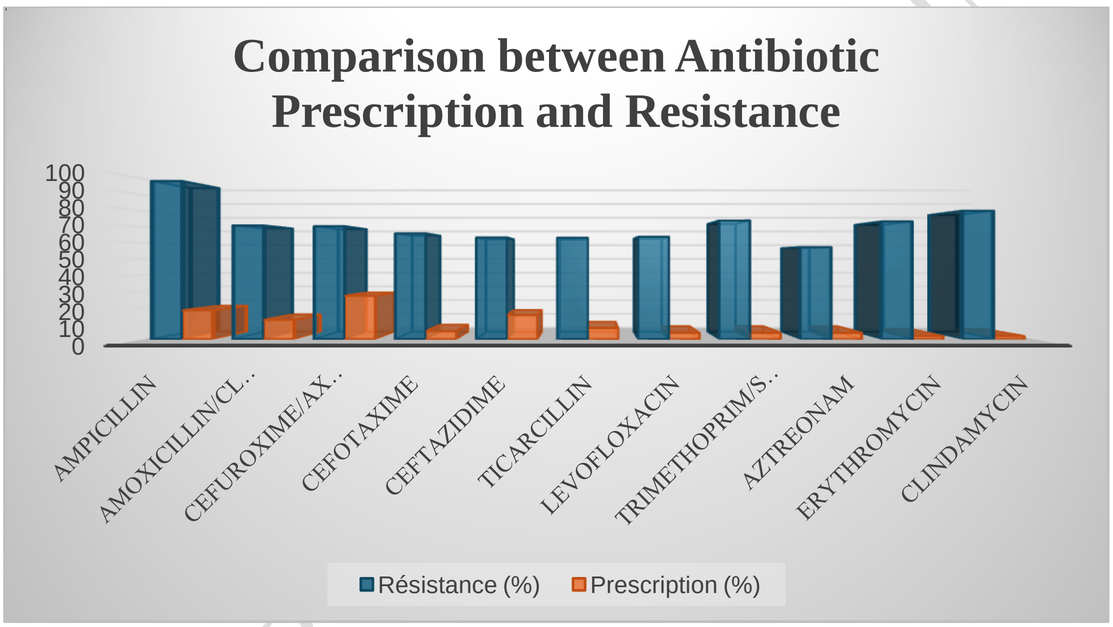


Figure 11. Susceptibility and resistance profiles to the most frequently prescribed antibiotics in the rural setting studied.

3.3. Genotypic characterization

The genetic profile of the isolates (Figure 12) revealed a predominance of CTX-M β-lactamases, detected in 39% of isolates (49/126; 95% CI [30.3–47.5]), representing the most frequently identified resistance mechanism. TEM/SHV genes, associated with resistance to penicillin's and first-generation cephalosporins, followed with a prevalence of 26% (32/123; 95% CI [18.2–33.8]). Carbapenemases producing genes (KPC, VIM, NDM, OXA-48) were detected in 15% of isolates (19/127; 95% CI [8.7–21.3]).

Less frequently detected mechanisms included other β-lactamases, observed in 9% of isolates (11/122; 95% CI [4.0–14.0]), and glycopeptide resistance mediated by vanA/vanB genes, identified in 4% of samples (5/125; 95% CI [0.6–7.4]). Finally, the mecA gene, responsible for methicillin resistance, showed a low prevalence of 3% (4/133; 95% CI [0.1–5.9]).

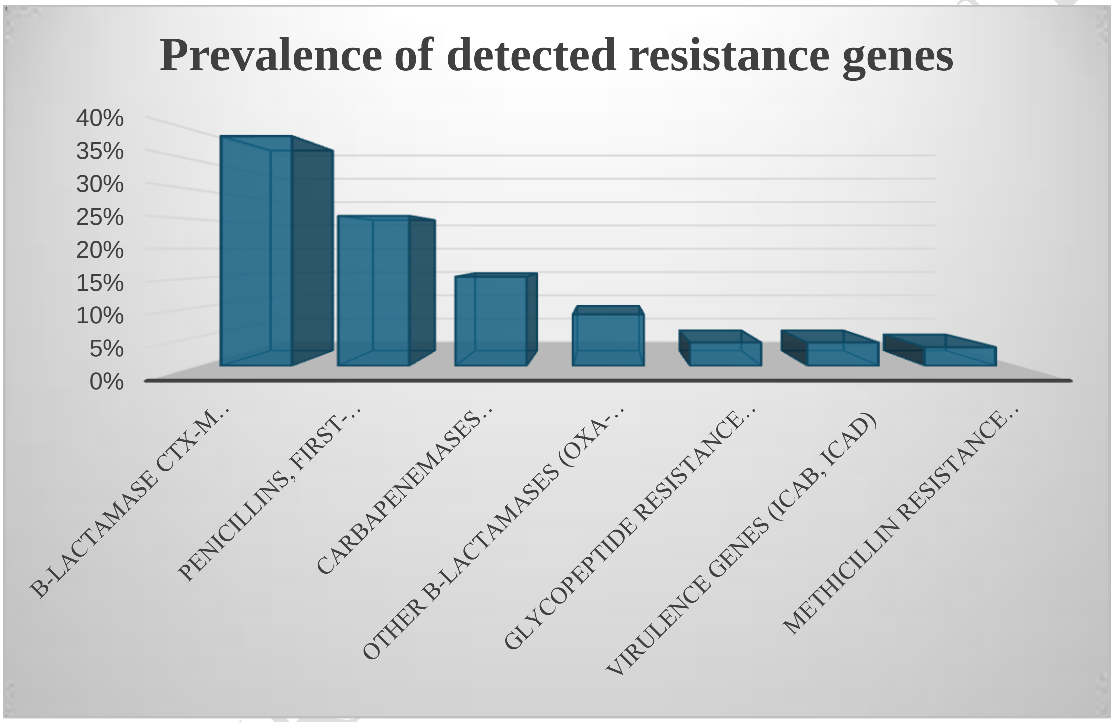


Figure 12. Frequency of identified antibiotic resistance genes.

3.4.Performance of on-site diagnostic testing

The overall analysis of the screening test (Table 7), using the VITEK® 2 antibiogram as the reference method, demonstrated a sensitivity of 80% (69/86; 95% CI [71.2–88.8]) and a specificity of 85% (73/86; 95% CI [75.0–91.9]). The positive predictive value (PPV) was high at 90% (77/86; 95% CI [82.4–95.1]), whereas the negative predictive value (NPV) was lower at 72% (62/86; 95% CI [61.6–80.9]). At the strain level, the detection sensitivity of MRSA agar reached 88% (7/8; 95% CI [52.9–97.8]) with a specificity of 94% (15/16; 95% CI [71.7–98.9]).

For VRE agar, performance was variable: specificity was excellent at 100% (3/3; 95% CI [43.9–100]), whereas sensitivity was more moderate at 75% (3/4; 95% CI [30.1–95.5]).

Diagnostic performance for multidrug-resistant *E. coli*, the most frequently identified species in this study, using ESBL and CPE agar was excellent, with both sensitivity and NPV of 100% (41/41; 95% CI [91.4–100]) and a specificity of 96% (22/23; 95% CI [87.3–100]). Similarly, for multidrug-resistant *K. pneumoniae*, specificity was perfect at 100% (5/5; 95% CI [48.0–100]), with a sensitivity of 82% (9/11; 95% CI [59.1–100]).

Detection of multidrug-resistant *Enterobacter* spp. showed lower sensitivity at 73% (8/11; 95% CI [46.4–89.3]) but maximal specificity at 100% (11/11; 95% CI [73.4–100]). Diagnostic performance for multidrug-resistant *Pseudomonas* spp. was very poor, with a sensitivity of 25% (2/8; 95% CI [7.1–59.1]) and a specificity of only 20% (1/3; 95% CI [3.6–62.5]). For multidrug-resistant *Salmonella* spp., the results are based on only two cases, and on a single case for *Acinetobacter*.

Table 7. Diagnostic performance parameters (DP) in on-site data collection.

Parameters	n	Se	Sp	PPV	NPV	DP
Overall value	86	80%	85%	90%	72%	84%
Methicillin-resistant *Staphylococcus aureus* (MRSA)	8	88%	94%	88%	94%	92%
Vancomycin-resistant *Enterococcus faecalis* and *Enterococcus casseliflavus* (VRE)	4	75%	100%	100%	75%	86%
Multidrug-resistant *Escherichia coli*	41	100%	96%	98%	100%	98%
Multidrug-resistant *Klebsiella pneumoniae*	11	82%	100%	100%	71%	88%
Multidrug-resistant *Enterobacter* spp.	11	73%	100%	100%	79%	86%
Multidrug-resistant *Pseudomonas* spp.	8	25%	20%	33%	14%	23%
Multidrug-resistant *Salmonella* spp.	2	100%	-	100%	-	100%
Multidrug-resistant *Acinetobacter* spp.	1	100%	-	100%	-	100%

Diagnostic performance = ((TP + TN) / (TP + TN + FP + FN)) × 100

TP = True Positives   TN = True Negatives   FP = False Positives   FN = False Negatives

3.5. Estimation of the cost of analysis in the study

Cost estimation showed that consumables accounted for the largest proportion of expenses, with a cumulative cost exceeding €11, dominated by the blood culture bottle. Labor and equipment costs remained low, reflecting limited depreciation and analysis time. Overall, the estimated total cost of a single analysis in our study was approximately €14.4, as presented in Table 8.

Table 8: Estimated cost of a microbiological analysis in the study

Category	Item / Description	Total Cost (€)
Consumables	Blood culture bottle (media, Petri dish, etc.)	10,09
	Minor consumables (pipettes, Bunsen burner…)	0,18
	Major consumables (autoclave, water distiller…)	0,95
Labor	Productive hours per analysis	0,24
Equipment	Incubator (depreciation)	0,20
	Usage duration (portion per analysis)	0,09
	Analysis capacity per month	0,30
	Electricity per analysis	0,05
Reference (%)	—	2,31
Total	—	14,41

4. Discussion

Blood Cultures

Our study evaluated the antibiotic resistance of bacteria responsible for bloodstream infections in Sikasso (Mali) using an initial screening on CHROMagar selective media, followed by confirmation through automated identification and susceptibility testing (VITEK 2). Bloodstream infections account for up to 29% of all infections in low- and middle-income countries (LMICs) [38].In our study, we observed a blood culture positivity rate of 29.13% (95% CI [25.35–33.23]), which is relatively high compared to the estimated African average of up to 15.5% [38],[39][40]. The study population comprised 23% children (0–17 years) and 77% adults (18–86 years). Females accounted for 39.8% (202/508; 95% CI [35.5–44.1]) and males for 32.5% (165/508; 95% CI [28.4–36.7]), while sex was not recorded in 27.8% of cases (141/508; 95% CI [23.9–

31.8]). One blood culture was collected per patient, with a contamination rate of 1.18%, which is below the CLSI recommended threshold (≤ 3%) and relatively low compared to rates reported in other countries, which may reach up to 10% (Iran, Ghana, South Africa) [34], [41],[42],[43], with a mean contamination rate of 7% (95% CI 6–7%) [44].Contaminants in our study were mainly skin or environmental bacteria, particularly coagulase-negative *Staphylococcus* species (*S. epidermidis, S. hominis, S. sciuri, S. lutrae*), as well as sporadic genera such as *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Aerococcus viridans*, *Pseudomonas stutzeri*, and *Clostridium* spp. [38], [43],[45], [46], [47].

Isolated bacterial strains

In our study, Enterobacteriaceae were isolated in 75.9% of cases (85/112; 95% CI [68.3–83.6]), while *staphylococci* and *enterococci* accounted for 18.8% (21/112; 95% CI [11.6–25.9]). A total of 6.3% (7/112; 95% CI [1.8–10.8]) of cases were caused by other bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp. More specifically, *E. coli* was the most frequently isolated pathogen (43.8%; 49/112; 95% CI [34.6–52.9]), followed by *Enterobacter* spp. (17.9%; 20/112; 95% CI [10.8–25.0]), *K. pneumoniae* (11.6%; 13/112; 95% CI [5.7–17.5]), and *Salmonella* spp. (1.8%; 2/112; 95% CI [0–4.2]). Other Gram-negative bacteria included *P. aeruginosa* (5.4%; 6/112; 95% CI [1.2–9.5]) and *A. baumannii* (0.9%; 1/112; 95% CI [0–2.6]). Among Gram-positive bacteria, *S. aureus* accounted for 15.5% of the isolated strains (17/112; 95% CI [8.5–21.8]), followed by *Enterococcus* spp. (3.6%; 4/112; 95% CI [0.1–7.0]). These results are comparable to those reported in a study conducted in Bamako (Mali) [48], where *Enterobacteriaceae* constituted the most frequently identified bacterial group (72.89%), followed by Gram-positive cocci (15.72%). The main bacterial species isolated in that study were *E. coli* (43%), *K. pneumoniae* (11.2%), *S. aureus* (4.3%), *P. aeruginosa* (3.1%), and *A. baumannii* (2.4%). In Rwanda [49], *Klebsiella* spp. accounted for 41% of Gram-negative isolates, followed by *Acinetobacter* spp. (15%) and *Pseudomonas* spp. (6.5%), while among Gram-positive cocci, *S. aureus* represented 50% and coagulase-negative *staphylococci* 35%. Conversely, a study conducted in Gabon[41], reported *S. aureus* as the predominant pathogen, followed by *E. coli, S. saprophyticus,* and *K. pneumoniae*. Similarly, a study from Gambia [50]identified *S. aureus* (41%), *Klebsiella* spp. (16%), other Enterobacteriaceae (13%), *Pseudomonas* spp. (8%), and *Acinetobacter* spp. (7%) as the most frequent pathogens among cases of bacteremia.

Contamination rate

The contamination rates of agar media observed, up to 1.99% (25/1253; 95% CI [1.35–2.94]), remain below the 3% threshold, indicating good control of aseptic conditions during manual preparation of the media. These results are consistent with quality standards recommended for microbiology laboratories, including those operating in resource-limited settings [51].

Such performance attests to the reliability of the manufacturing process and the applied quality control system, which are essential for controlling culture conditions and obtaining reliable microbiological results. In this study, the manufacturing process and the implemented quality control system demonstrate satisfactory reliability, reflecting good control of aseptic conditions. Regular maintenance of these verification procedures remains essential to ensure the reproducibility of results and the validity of bacterial cultures, in accordance with international recommendations. An additional option to further strengthen these controls would be to perform a parallel growth test using tubes containing a solid agar medium, thereby confirming the multiplication capacity of the strains and improving the robustness of quality control. This approach is consistent with good practices described for the evaluation of the microbiological performance of culture media (ISO 11133:2014) [52], [53].

Performance of the simplified method

In our study, the sensitivity of the media for detecting multidrug-resistant bacteria (MDR) varied according to the bacterial species. It was very high for methicillin-resistant *S. aureus* at 88% (7/8; 95% CI [68.8–100]), close to that reported in East Africa (89%)[54], [55]. For VRE *enterococci*, sensitivity remained high at 75% (3/4; 95% CI [32.6–100]), whereas another agar, CHROMID VRE, demonstrated a sensitivity of 96.9% [56]. The results were excellent for *E. coli* at 100% (41/41; 95% CI 100%) in our study, as also reported in Botswana (100%) [57]. The sensitivity for *K. pneumoniae* was 82% (9/11; 95% CI [62.4–100]) in our study, which is lower than that observed in the Botswana study (100%)[57]. Likewise, the sensitivity for *Enterobacter* spp. was 73% (8/11; 95% CI [48.7–97.3]), lower than the overall performance reported in Uganda (100%) [58]. This discrepancy may be explained by differences in the species tested, bacterial loads, inoculation/reading conditions, or resistance mechanisms not producing ESBL (e.g., AmpC, porins/efflux), which are less well detected on ESBL media [59], [60]. A low sensitivity was observed in our study for *Pseudomonas* spp. at 25% (2/8; 95% CI [0–55]). In contrast, one study reported much higher sensitivities, reaching 91% with chromogenic *Pseudomonas aeruginosa* agars (PACA) and CHROMagar™ *Pseudomonas*, 85% with

CHROMID®*P. aeruginosa*, and 83% with MacConkey agar [61].This gap may be explained by the fact that our protocol mainly used specific media targeting three pathogen groups (*Staphylococcus*, *Enterococcus*, and Enterobacteriaceae), thereby limiting optimal detection of *Pseudomonas* spp. In addition, Enterobacteriaceae and other opportunistic bacteria were mainly observed on ESBL/CPE media and on the ORI control, which may have reduced the visibility or growth of *Pseudomonas* spp. in our experimental setup, leading to an apparently lower sensitivity.

Overall, specificity was high in our study, reaching 94% for *S. aureus* (15/16; 95% CI [71.7–98.9]), which is close to the performance reported for chromogenic media such as MRSA ID (98% at 24 h) [62], [63]. The 100% specificity (3/3; 95% CI [43.9–100]) observed for VRE in our study is noteworthy. Although poorly documented in the African context, it is supported by studies from other countries reporting a specificity of 99% for chromogenic VRE media compared with an E-test reference standard [63], [64]. The specificity observed for *E. coli* (96%; 22/23; 95% CI [87.3–100]), *K. pneumoniae* (100%; 5/5; 95% CI [48–100]), and *Enterobacter* spp. (100%; 11/11; 95% CI [73.4–100]) is comparable to the recent study conducted in Botswana, which reported a specificity of 91.2% (95% CI: 88.4–93.3) for *E. coli* and 88.1% (95% CI: 83.2–92.1) for the KEC group (*Klebsiella–Enterobacter–Citrobacter*) [57].In contrast, *Pseudomonas* spp. showed a very low specificity (20%), far below that reported for chromogenic media, which nevertheless remains only moderate overall (60–75%) [61].

The PPV was high for most species in our study, reaching 88% (7/8) for *S. aureus*. This value lies between those reported in Uganda using DNase identification (96%, higher), Mannitol Salt Agar (83%, lower), and the tube coagulase test alone (54–50%, markedly lower) [65]. It was even higher for VRE at 100% (3/3; 95% CI [43.9–100]), exceeding that reported in Egypt for chromogenic VRE medium (91.7%) [64].PPV was also excellent for *E. coli* at 98% (41/42; 95% CI [93–100]), in agreement with a Belgian study using Brilliance ESBL agar that reported a similar PPV [66]. Likewise, a perfect PPV was observed in our study for *K. pneumoniae* (100%; 9/9; 95% CI [66–100]) and *Enterobacter* spp. (100%; 8/8; 95% CI [67.6–100]), which is higher than that reported in the Botswana study where CHROMagar ESBL showed a PPV of 88.1% for the KEC group, thereby confirming its reliability as a screening tool in Africa [57].In contrast, the PPV for *Pseudomonas* spp. was very low (33%). However, a recent study conducted outside the African context reported much higher PPVs, with PACA medium reaching 95%, CHROMID P. aeruginosa 86%, and CHROMagar™ *Pseudomonas* only 56% [61].

The high NPV for *S. aureus* observed in our study (94%; 15/16; 95% CI [71.7–98.9]) is supported by data from studies using chromogenic media for MRSA, which reported an estimated NPV of 99.8% [67]. Our NPV of 100% for *E. coli* (22/22; 95% CI [85.3–100]) is corroborated by another study that also reported an NPV of 100% for KC-ESBL medium in the detection of ESBL-producing Enterobacteriaceae[68]. The NPV observed for *K. pneumoniae* in our study (71%; 5/7; 95% CI [37.9–100]) remains more modest than that reported for Brilliance ESBL agar, which reached 96.9% [69].Similarly, the moderate NPV of 79% (11/14; 95% CI [52.4–92.9]) observed for *Enterobacter* spp. in our study is lower than that reported for KC-ESBL medium (100%) [68]. In contrast to the exceptionally low NPV observed in our study for *Pseudomonas* spp. (14%; 1/7; 95% CI [2.6–51.3]), an international comparative study reported much higher NPVs for this pathogen; for example, two chromogenic media achieved NPVs of 90% and 96% for P. aeruginosa [70]. Finally, although the sample sizes were small, Salmonella spp. and Acinetobacter spp. showed high NPVs (100%, with 2 and 1 isolates, respectively). These performances are illustrated by CHROMagar *Salmonella* Plus (no false positives detected) [71]and CHROMagar Acinetobacter with CR102 supplement (NPV of 100%) [72].Overall, the diagnostic performance of the simplified method used in this study was high, reaching 84% (72/86; 95% CI [74.5–90.0]). Detailed overall analysis showed a sensitivity of 80% (69/86; 95% CI [69.3–86.3]) and a specificity of 85% (73/86; 95% CI [75.8–90.9]), with a PPV of 90% (62/86; 95% CI [81.3–94.4]) and an NPV of 72% (62/86; 95% CI [60.6–79.5]). These results indicate robust diagnostic performance, suggesting that the method effectively identifies resistant strains while limiting over-detection.

Cost of analysis

The total cost of our simplified method is estimated at €14.41 per test, including all components (consumables, labor, depreciation, etc.). This cost appears particularly competitive when compared with the costs reported for blood cultures in resource-limited settings. Indeed, several recent studies conducted in Africa and other low- and middle-income countries have reported costs of up to €21.51 per test [73], [74], [75]. In Malian referral hospitals (e.g., university hospitals or the Pasteur Institute of Mali), the actual cost of a standard blood culture is generally estimated at around €22, whereas in regional hospitals (Sikasso, Kayes) this cost can reach €28 because of additional logistical expenses and limited purchasing capacity [75].Thus, our method represents a cost reduction of up to 50%, depending on the type of facility.

This difference is particularly important for peripheral centers, which are often not equipped to perform blood cultures and could integrate this diagnostic approach at an affordable cost for patients. It would also spare patients the expense of traveling to regional hospitals or to the capital for diagnostic confirmation.

Antimicrobial susceptibility testing

The isolated bacteria highlight an alarming prevalence of resistance to several antibiotics, with a high resistance profile among Gram-negative strains. Our study shows that 94% (32/34; 95% CI [80.9–98.4]) of isolates were resistant to amoxicillin/clavulanic acid, followed by 91% (31/34; 95% CI [77.0–97.0]) resistant to cefotaxime and cefepime, and 88% (30/34; 95% CI [73.4–95.3]) resistant to ceftazidime.

The prevalence of ESBL-producing strains was 37% for *E. coli* (15/41; 95% CI [23.6–51.9]) and 36% for *K. pneumoniae* (4/11; 95% CI [15.2–64.6]). These two species therefore play a major role as reservoirs of resistance in both community- and hospital-acquired infections. In Mali, a study conducted in Bamako showed that 61.8% of Enterobacteriaceae isolates from blood cultures were ESBL producers, with particularly high rates among *K. pneumoniae* (71%) and *E. coli* (73%) [76].

The presence of such high proportions suggests strong antibiotic selective pressure on these species, which are frequently targeted by β-lactams, thereby promoting the selection and spread of resistant strains. However, *K. aerogenes* (formerly known as*Enterobacter aerogenes*) showed in our study a proportion of ESBL-producing strains of 55% (6/11; 95% CI [28.0–78.7]), higher than that observed for *E. coli* and *K. pneumoniae*, but lower than the rates reported in Bamako[76]. In contrast, no ESBL-producing strains were detected among *Salmonella* spp. and *Pseudomonas* spp. This absence may be explained by lower exposure to certain antibiotics in *Salmonella* infections, which are often community-acquired, and by the multifactorial intrinsic resistance characteristic of *Pseudomonas* spp. These findings underscore the importance of a targeted approach to antimicrobial resistance surveillance that considers species-specific characteristics, in order to optimize empirical treatment strategies and improve antimicrobial stewardship.

Resistance among Gram-positive bacterial strains reveals a worrying situation, particularly with regard to glycopeptides, which are often considered last-resort antibiotics. Indeed, our study shows that 83% of the strains are resistant to vancomycin and teicoplanin (including one case of

VRSA). Such resistance is rare but serious, as it considerably limits the available therapeutic options.

Antibiotic resistance among Gram-positive cocci is a major concern, particularly for *S. haemolyticus* and *S. aureus* in both hospital and community settings, as reported in a Malian study conducted in Bamako [48]. Our study reveals trends similar to those reported in other African contexts. The very high proportion of vancomycin-resistant strains observed (83%) is comparable to data from a meta-analysis conducted in Ethiopia, which estimated the prevalence of vancomycin-resistant *S. aureus* (VRSA) at 14.5% (95% CI: 11.6–17.4%) [48], [77]. The same study also reported a mean prevalence of 14.8% (95% CI: 8.7–24.3%) for vancomycin-resistant *Enterococcus* (VRE) in various African clinical samples [48], [77], with notably high resistance to vancomycin and other commonly used antibiotics. Methicillin resistance increased from 28% to 68% [77], and with regard to vancomycin, resistance rates of 23.8% for *S. haemolyticus* and 14.28% for *S. aureus* have been reported in the literature [48].

Our results suggest increasing selective pressure on carbapenems, probably related to their intensified use following the failure of first- and second-line antibiotics. This trend highlights a worrying evolution of resistance among Gram-negative bacteria, particularly with the confirmation of carbapenemase-producing Enterobacteriaceae (CPE) strains in our study. In parallel, the high level of resistance to glycopeptides (83%) observed among Gram-positive bacteria underscores the need to restrict the use of critical antibiotics such as vancomycin and teicoplanin. Unregulated use of these agents could promote the emergence of multidrug-resistant strains such as VRE and VRSA. It is therefore urgent to strengthen microbiological surveillance, implement appropriate antibiotic stewardship protocols, and promote rational antibiotic use in order to curb the spread of these resistant pathogens. These findings support the implementation of local and national strategies to combat antimicrobial resistance, integrating routine microbiology, pharmacovigilance, and continuous training of prescribers.

Frequently prescribed antibiotics

The analysis of prescribing patterns confirms a substantial reliance on broad-spectrum antibiotics, particularly third-generation cephalosporins and fluoroquinolones, despite the high resistance rates observed for several of these agents. Ceftriaxone was prescribed in 16% of cases (16/100; 95% CI [10.1–24.4]) and exhibited a resistance rate of 84%, while amoxicillin/clavulanic acid accounted for 11% of prescriptions (11/100; 95% CI [6.3–18.6]) with an 80% resistance rate. Similarly, cotrimoxazole was prescribed in 12% of cases (12/100; 95%

CI [7.0–19.8]) and showed a resistance rate of 66%, whereas ciprofloxacin, used in 5% of cases (5/100; 95% CI [2.1–11.2]), demonstrated 51% resistance. Ceftazidime was prescribed in 7% of cases (7/100; 95% CI [3.4–13.9]) and exhibited a resistance rate of 57%. Levofloxacin accounted for 4% of prescriptions (4/100; 95% CI [1.6–9.8]) with a resistance rate of 37%, consistent with trends reported in other Sub-Saharan African countries[78].In contrast, gentamicin, although infrequently prescribed (2%; 2/100; 95% CI [0.5–7.0]), retained relatively high susceptibility (73%), suggesting potential underutilization given its favorable microbiological profile. Furthermore, frequently prescribed agents such as azithromycin (18%; 18/100; 95% CI [11.7–26.7]), cefixime (15%; 15/100; 95% CI [9.4–23.0]), and clarithromycin (2%; 2/100; 95% CI [0.5–7.0]) were not evaluated in the susceptibility analysis, limiting a comprehensive assessment of their therapeutic appropriateness.Overall, these findings highlight a concerning mismatch between empirical prescribing practices and local bacterial resistance patterns, underscoring the urgent need to strengthen antimicrobial stewardship strategies in the Malian context, in line with the priorities of the World Health Organization Global Action Plan on Antimicrobial Resistance[79].This is consistent with reports from other Sub-Saharan African countries, where fluoroquinolones demonstrate only moderate activity, with susceptibility rates of 46% for levofloxacin and 37% for ciprofloxacin[80]. In African settings such as Ethiopia and the Democratic Republic of Congo, gentamicin is used in only 10.7% and 5.6% of prescriptions, respectively, whereas third-generation cephalosporins account for more than 20% of prescriptions[81]. This distribution reflects a marked preference for broad-spectrum antibiotics, likely influenced by several structural constraints, including limited diagnostic capacity, sometimes insufficient clinical follow-up to allow therapeutic adjustment, as well as local resistance patterns and the predominance of empirical prescribing practices, rather than constituting unjustified overuse in the strict sense[81].In contrast, in our study, resistance to meropenem remains relatively low (15%), making it one of the few antibiotics that are still largely effective. However, the presence of carbapenem-resistant strains is of concern, as it suggests a possible emergence of carbapenemase-producing organisms and warrants close surveillance. These findings are consistent with trends reported in the literature from Mali [48], where *E. coli* exhibited resistance rates of 100% to amoxicillin, 92.6% to amoxicillin/clavulanic acid, and 81% to ofloxacin, while *K. pneumoniae* showed resistance rates of 88.8% to amoxicillin and 78.26% to amoxicillin/clavulanic acid. Other studies [82], report that ESBL-producing Enterobacteriaceae are resistant to cotrimoxazole (84%), ciprofloxacin (81.82%), and gentamicin (61.76%), whereas amikacin remains effective in 57.14% of cases. Our results are consistent with this Malian study. However, with regard to A. baumannii, data from the same study [82], reveal complete resistance (100%) to ciprofloxacin and a higher resistance rate to cefotaxime (83.33%) than that observed in our study (70%).

Our results are similar to those reported in studies conducted in neighboring countries, for example at the National Hospital of Niamey (Niger), where 100% of *E. coli* isolates were reported to be resistant to amoxicillin and clavulanic acid and 85.1% to ceftriaxone [83]. It has also been reported that almost all *E. coli* strains were resistant to the amoxicillin–clavulanic acid combination in a study conducted in Ouagadougou (Burkina Faso) [84].

Automated Antimicrobial Susceptibility Testing

Automated antimicrobial susceptibility testing using the VITEK 2 system confirms high levels of resistance to β-lactam antibiotics among Gram-negative bacilli, particularly *K. pneumoniae* and *E. coli,* with near-complete resistance to ampicillin (100% and 95%, respectively), consistent with rates reported in Africa (90–100%)[85]. Resistance to penicillin–β-lactamase inhibitor combinations remain a major concern, reaching 92% in *E. coli* and 50% in *K. pneumoniae*, comparable to data from Mali and Morocco [48],[86]. Third-generation cephalosporins exhibit high resistance rates in *E. coli* (≥79%) and *K. pneumoniae* (≈50%), suggesting a high prevalence of ESBL-producing strains, as reported in Cameroon and Ethiopia[87], [88]. Carbapenem resistance remains clinically significant, with 17% resistance to ertapenem and 8% to meropenem in *K. pneumoniae*, indicating the emergence of carbapenemase-producing strains previously described in Egypt and East Africa [89],[90]. Finally, the high resistance to fluoroquinolones (ciprofloxacin: 67% in *E. coli)* and trimethoprim–sulfamethoxazole (90% in *E. coli)* further limits first-line therapeutic options, as observed in several African studies[91], [92], [93].

Among Gram-positive bacteria, our results show an oxacillin resistance rate of 29% in *S. aureus*, suggesting the circulation of methicillin-resistant strains (MRSA), although this rate is lower than those reported in Mali (100%) [48] and in Niamey (83%) [83].Resistance to macrolides–lincosamides (erythromycin 47%, clindamycin 60%) indicates a significant spread of macrolide–lincosamide–streptogramin (MLS) phenotypes, as previously observed in Morocco where MRSA prevalence ranged from 1.6% to 31.1%[86]. Moderate resistance to glycopeptides, with 27% resistance to vancomycin and 40% to teicoplanin in *S. aureus,* is particularly concerning and suggests the emergence of VISA/GISA strains, thereby limiting first-line therapeutic options [83]. High resistance to tetracycline (73%) is consistent with data from Bamako (100%) [48]. In contrast, some antibiotics such as ciprofloxacin (7%), chloramphenicol (7%), and mupirocin (14%) retain satisfactory activity (resistance ≤14%) [48].

Among *Enterococcus* spp., a multidrug-resistant profile is characterized by uniform resistance (100%) to erythromycin, clindamycin, minocycline, and trimethoprim–sulfamethoxazole, as well as concerning resistance to vancomycin and teicoplanin (50% each), suggesting the presence of vancomycin-resistant enterococci (VRE). This trend is consistent with data from Uganda, where enterococci show high resistance rates to macrolides and tetracyclines[94]. Tigecycline, with a more moderate resistance rate (33%), remains one of the few partially effective therapeutic options.

Polymerase Chain Reaction (PCR) Analysis

PCR analyses reported in a study conducted in Indonesia [95]showed that blaCTX-M-15 is the most frequent ESBL gene in *Klebsiella pneumoniae,* detected in 89.4% of isolates (84/94). The blaSHV gene was identified in 39.4% of strains, of which 33 also co-harbored blaCTX-M-15[95]. In addition, blaTEM was detected in 46.8% of isolates (44/94), although all corresponded to TEM-1 variants, which are not ESBL producers[95].Our results confirm the high prevalence of blaTEM/SHV (26%) and blaCTX-M (39%) genes detected in our isolates, a trend similar to that reported in several studies from China and Tehran [96], [97]. These genes, which are involved in resistance to β-lactams (penicillins and cephalosporins), highlight the importance of strengthened hospital surveillance to limit the spread of multidrug-resistant strains in the study area. The high prevalence of blaCTX-M genes (39%) in our series reflects an accelerated circulation of ESBL-producing strains, particularly of the CTX-M-15 type, which has been documented at rates exceeding 80% in several African hospitals, notably in Nigeria and Ethiopia[98]. These genes may facilitate the rapid dissemination of hyper-resistant clones in hospital settings, as illustrated by the dominance of blaCTX-M-15 among *K. pneumoniae* isolates in Ethiopian studies, indicating a high risk of nosocomial outbreaks[99]. The notable presence of blaTEM and blaSHV genes (26%) also indicates the spread of classical ESBLs, thereby compromising the efficacy of penicillins and third-generation cephalosporins. These resistance mechanisms severely limit empirical treatment options, placing carbapenems as last-resort agents, which are often available but subject to increasing selective pressure.

The detection of carbapenem resistance genes (NDM, VIM, OXA-48, KPC), although at a relatively low prevalence (15%), remains a major concern due to their association with high-level resistance to carbapenems, which drastically limits therapeutic options [100]. In sub-

Saharan Africa, the emergence of NDM and OXA-48 has been reported at variable frequencies (ranging from 2% to >10% depending on the region), particularly in intensive care units[101]. Even at low prevalence, the circulation of these genes among Enterobacteriaceae represents an alarming signal and warrants the implementation of strict microbiological surveillance and infection control measures to prevent their dissemination.

The identification of the mecA gene in multidrug-resistant *S. aureus* as well as vanA and vanB genes in *Enterococcus faecalis/casseliflavus* corroborates recent data on the dissemination of these resistance determinants in hospital-acquired infections[102]. In our study, although the prevalence of MRSA strains was low, they nonetheless harbored major resistance mechanisms, notably mecA. Although still infrequent, the detection of even a single vanA or vanB gene in E*nterococcus* spp. (4%) is alarming, as these determinants confer high-level resistance to vancomycin, a last-resort antibiotic. Their spread in hospital settings could lead to therapeutic dead ends for certain infections. A pooled analysis of African data reports a mean prevalence of vancomycin-resistant enterococci (VRE) of 26.8% (95% CI: 10.7–43.0%) across various clinical settings, with peaks of up to 75% in South Africa [103]. These findings, even when isolated, call for strengthened infection prevention measures (targeted screening, isolation, strict disinfection) and antibiogram-guided therapy to prevent the dissemination of these resistance mechanisms within hospital wards.

According to the literature, the frequencies of vanA and vanB genes were relatively low (15.8% and 7.9%, respectively) and were detected only in E. casseliflavus/gallinarum species, which are intrinsically resistant to vancomycin[94]. The detection of a vanA gene in a vancomycin-resistant *S. aureus* (VRSA) isolate in our study is particularly concerning, as VRSA remains rare but extremely difficult to treat, requiring strict surveillance and isolation measures to prevent its spread.

Although chromogenic media provide rapid, presumptive visual identification of bacteria, they are not fully reliable for all species. Some strains, particularly rare or fastidious organisms, may fail to express the target enzyme, leading to false-negative results. In addition, colony color may be influenced by metabolic variations or culture conditions, potentially causing misinterpretation and misidentification of closely related species[104]. In mixed flora samples (co-infection, contamination, polymicrobial infections), visual presumptive identification may mask minority pathogens, thereby compromising overall diagnostic sensitivity [105]. Therefore, chromogenic media should ideally be complemented by confirmatory methods (biochemical testing, mass spectrometry, PCR) to ensure accurate identification. Nevertheless, rapid communication of the presumptive pathogen genus to clinicians allows earlier optimization of antimicrobial therapy, thereby improving clinical relevance. Several studies have shown that early bacterial identification enhances therapeutic adjustment and reduces inappropriate antibiotic prescriptions[106].

5. Conclusion

In conclusion, this study highlights the capacity of in situ detection tests using CHROMagar media for the isolation of resistant bacterial strains in primary healthcare centers. The strains collected can subsequently be submitted for confirmatory analyses in a reference laboratory, usually located in the capital. While emphasizing the need for regular quality control to reduce diagnostic errors and improve surveillance of multidrug-resistant bacteria, we recommend integrating this simplified method into routine laboratory practice in rural settings.

The recommendations of this study reinforce the importance of strict hygiene precautions to prevent bacterial transmission and the use of alternative therapeutic options for the management of resistant infections identified in this work, while maintaining surveillance of resistance genes to limit the dissemination of multidrug-resistant strains. Co-infections may complicate antibiotic selection due to multiple resistance mechanisms, thereby increasing the risk of morbidity, particularly among immunocompromised patients or those in intensive care. These findings may also reflect a history of prolonged antibiotic exposure or extended hospitalization in some patients.

Rational use of β-lactam antibiotics is essential and should be guided by prudent prescription practices to limit the selection and spread of resistance. The presence of carbapenemase genes requires avoidance of carbapenems except as a last resort, favoring alternative therapeutic options such as colistin, tigecycline, and fosfomycin. These genes are also associated with biofilm formation, further enhancing bacterial persistence and treatment failure. A rigorous antimicrobial stewardship approach, combined with infection prevention measures, is therefore essential to curb the spread of resistance.

Overall, this evaluation demonstrates that CHROMagar is a valuable initial screening tool, particularly for major Enterobacteriaceae (*E. coli, K. pneumoniae),* but that it has limitations in sensitivity and specificity depending on the species. Its use in resource-limited settings is justified, provided that it is supported by a robust confirmatory testing strategy.

Ethics Statement

Blood sample collection for blood cultures and the associated analyses was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with applicable regulations. Biological samples were obtained under protocols approved by the local ethics committee of the National Ethics Committee for Health and Life Sciences (CNESS), in particular under Program No. 2022-138_/MSDS-CNESS dated 18/11/2022.

Program No. 2022-138 involved the collection of blood samples inoculated into blood culture bottles, followed by incubation and subculture on chromogenic media to observe bacterial growth. Pathogenic colonies were collected and sent for comprehensive analysis to the Clinical Microbiology Laboratory of the University Hospital of Liege for bacterial species identification and resistance gene detection.

All participants provided informed consent in the form of a questionnaire completed by the attending physician prior to patient inclusion in the study, that is, before any sample collection. The approved procedures included standardized processing and storage of samples (colonies on agar and blood culture broth frozen at −20 °C) to ensure sample integrity and traceability. All stages, from sample collection to data use, complied with requirements for confidentiality and protection of personal data.

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ANNEXES

Tableau 2 : Différents primers utilisés

Gènes	Forward	Reverse	pb
bla_BEL	CGA CAA TGC CGC AGC TAA CC	CAG AAG CAA TTA ATA ACG CCC	448
bla_ADCb	GTA CCT CAA TTT ATG CGG RCA ATA C	TGC GYT CTT CAT TTG GAA TAC G	1059
OXA 1	GGCACCAGATTCAACTTTCAAG	GACCCCAAGTTTCCTGTAAGTG	564
CTX-M	ACC GCG ATA TCG TTG GT	CGC TTT GCG ATG YGC AG	550
CTX-9	TCAAGCCTGCCGATCTGGT	TGATTCTCGCCGCTGAAG	561
CTX-2	CGTTAACGGCACGATGAC	CGATATCGTTGGTGGTGCCAT	404
CTX-1	TTAGGAAATGTGCCGCTGTA	CGATATCGTTGGTGGTACCAT	688
SHV	AGCCGCTTGAGCAAATTAAAC	ATCCCGCAGATAAATCACCAC	713
TEM	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	800
KPC	TCG CCG TCT AGT TCT GCT GTC TTG	ACA GCT CCG CCA CCG TCA T	353
OXA 48	ATG CGT GTA TTA GCC TTA TCG	CAT CCT TAA CCA CGC CCA AAT C	265
VIM	TGT CCG TGA TGG TGA TGA GT	ATT CAG CCA GAT CGG CAT C	437

NDM	ACT TGG CCT TGC TGT CCT T	CAT TAG CCG CTG CAT TGA T	603
MEC A	AAAATCGATGGTAAAGGTTGGC	AGTTCTGGAGTACCGGATTTGC	533
FOX 1	CTACAGTGCGGGTGGTTT	CTATTTGCGGCCAGGTGA	162
VAN A	CATGACGTATCGGTAAAATC	ACCGGGCAGRGTATTGAC	885
VAN B	CATGATGTGTCGGTAAAATC	ACCGGGCAGRGTATTGAC	885
VAN C	CTCCTACGATTCTCTTG	CGAGCAAGACCTTTAAG	800
ica B	AGAATCGTGAAGTATAGAAAATT	TCTAATCTTTTTCATGGAATCCGT	900
ica D	ATGGTCAAGCCCAGACAGAG	AGTATTTTCAATGTTTAAAGCAA	198