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Assessment of CHROMagar Culture Media for the Rapid Detection of Antibiotic-Resistant Strains (*Staphylococcus Aureus, Enterococcus Sp.*, and *Enterobacteriaceae*) under Suboptimal Storage

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Abstract

Antimicrobial resistance (AMR) is a major global public health threat, potentially affecting up to 82% of bloodstream infection cases depending on the country. Rapid detection of multidrug-resistant bacteria is essential for effective management. Among the most frequently reported priority pathogens are *Escherichia coli*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Salmonella spp.*, for which early and targeted identification is crucial to guide appropriate treatment.This study assesses the performance of chromogenic media in detecting and presumptively identifying methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), and beta-lactam-resistant *Enterobacteriaceae* (ESBL), including carbapenem-resistant *Enterobacteriaceae* (CPE). The CHROMagar MRSA, VRE, ESBL, and mSuperCARBA media were evaluated using known resistant strains and American Type

Culture Collection (ATCC) reference strains, comprising 3 strains of *Staphylococcus spp*., 10 strains of *Enterobacteriaceae*, and 4 strains of *Enterococcus spp.* Colony coloration were interpreted based on the manufacturer's guidelines.

Under optimal conditions, the ESBL, mSuperCARBA, and VRE media demonstrated 100% sensitivity and specificity for detection, while the MRSA medium exhibited 100% sensitivity but only 79% specificity. Under suboptimal conditions (storage at 20 degrees Celsius (°C) and 35°C in darkness, and 20°C under light), the ESBL, mSuperCARBA, and MRSA media maintained 100% sensitivity, though VRE sensitivity dropped to 78% under 20°C with light exposure. Specificity for ESBL, mSuperCARBA, and VRE remained at 100% in these conditions, while MRSA specificity decreased to 52%.

In summary, the tested CHROMagar media effectively detect

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resistant bacteria with high sensitivity and specificity under optimal conditions. However, MRSA and VRE media exhibit variability in performance under suboptimal storage conditions.

Keywords: MRSA, VRE, ESBL-producing *Enterobacteriaceae*, CPE, CHROMagar medium

Introduction

Since the first reported resistance of *Staphylococcus aureus* in 1961, the global burden of antimicrobial resistance (AMR) has continuously increased and is associated with higher morbidity and mortality in hospital settings [1,2]. Today, the emergence and spread of carbapenem-resistant *Enterobacteriaceae* is a global public health problem [3]. Based on predictive statistical models, the number of deaths associated with bacterial resistance in 2019 is estimated at 4.95 million (confidence interval [3.62–6.57]), among which 1.27 million (95% UI 0.911– 1.71) deaths are directly attributable to this resistance [4]. The diagnosis of resistant bacteria is crucial for ensuring effective patient management and adequate surveillance at local and national levels [5]. To optimize antibiotic therapy, the clinician must confirm or rule out the existence of an infection and, if present, determine the level of antibiotic susceptibility [6]. To meet this requirement, the clinical microbiology laboratory relies on recommendations issued by scientific committees such as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and/or the American Clinical & Laboratory Standards Institute (CLSI) (SFM, 2010) [7,8]. Conventional microbiological diagnostic protocols based on culture are often time consuming and expensive ("CASFM," 2022) [9,10]. To ensure quality in clinical bacteriology laboratories, it is essential to have a well-functioning network of reference laboratories, to enable effective resistance control. However, resource-limited regions face infrastructural, technical, and behavioral challenges in implementing clinical bacteriology [11]. In low- and middle-income countries, applying these protocols while adhering to required standards is particularly difficult, especially due to issues related to the maintenance of medical equipment and insufficient budget allocations by governments for public health emergencies, which also impacts reference centres (Ministère de la santé et de l'hygiène publique, Mali, 2016) [12]. It is also challenging to find equipment and supplies suitable for resource-limited countries. Furthermore, environmental conditions often damage electronic equipment and medical supplies. The quality of diagnostics is also compromised due to a lack of local production and poor chain supplies. Many diagnostic kits require cold storage, which is difficult to maintain under these circumstances [11].

Louis Pasteur was the first to culture a bacterium reproducibly in 1860 through the development of the first artificial culture medium [13]. Culture media are divided into several types, each with a specific role in isolating and identifying bacteria: non-selective media, such as blood agar, allow the growth of most bacteria and provide an overview of the pathogens present in a clinical sample, while selective media, containing inhibitory agents, limit the growth of non-target bacteria and promote the isolation of specific strains, such as those detected by CHROMagar MRSA for methicillin-resistant *Staphylococcus aureus* or mSuperCARBA for carbapenemase-producing *Enterobacteriaceae*. Differential media, on the other hand, allow bacteria to be distinguished based on visible biochemical characteristics, thus facilitating rapid identification [10]. Since the development of the first chromogenic medium by Alain Rambach in 1979, these media have become widely used in bacteriology, and the combined use of multiple chromogenic substrates only emerged in the 1990s, gradually expanding thereafter [14].

Over the years, some manufacturers have proposed chromogenic media allowing rapid preliminary identification of certain bacterial species directly from the primary culture of samples, particularly in the case of urine culture [15]. The principle of chromogenic media, whether selective or not, is based on the presence of one or more chromogenic substrates in the medium, whose degradation by specific bacterial enzymes releases a visible chromophore ("Milieu chromogene," 2018) [16]. Chromogenic media simplify protocols and improve diagnostic speed [10]. Unlike classical media, which often require obtaining a pure bacterial culture and a minimum delay of 48 to 72 hours, chromogenic media allow for accelerated preliminary and presumptive identification, generally within 18 to 24 hours [6].

The objective of this study is to validate the detection of four specific bacterial phenotypes, namely methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), extendedspectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, and carbapenem-resistant *Enterobacteriaceae* (CPE), using CHROMagar chromogenic media. Additionally, this study aims to demonstrate that the culture medium powder has a long shelf life and does not require refrigerated storage.

Materials and Methods

The study was conducted over 12 weeks to evaluate four CHROMagar media (MRSA, VRE, ESBL, mSuperCARBA). These tests were carried out in the microbiology laboratory of the University Hospital Centre of Liège (CHU Liège).

Reference strains

Duly identified bacterial strains were used to evaluate the four study media. All incubations were conducted at 35°C under aerobic conditions (not in CO_2). The seventeen strains used included the following media:

MRSA medium: Methicillin-resistant *Staphylococcus aureus*, characterized by the National Reference Centre

(CNR) Brussels Erasme Free University of Brussels (ULB) via Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF) and Value, Integrity, Teamwork, Excellence and Knowledge (VITEK), resistant to oxacillin, tetracycline, and fusidic acid. Pink colonies were observed after overnight incubation at 35°C.

- *Staphylococcus epidermidis*, identified by the microbiology laboratory of CHU Liège using MALDI-TOF
- and VITEK, resistant to oxacillin, tobramycin, erythromycin, clindamycin, tetracycline, and fusidic

acid. Absence of colony growth or the presence of non-pink colonies was observed after overnight incubation at 35°C.

- Methicillin-sensitive *Staphylococcus aureus* ATCC 29213 sub *sp. aureus* Rosenbach, a reference strain commonly used for antibiotic susceptibility testing, resistant to penicillin. No colony growth was observed after overnight incubation at 35°C.

VRE medium: *Enterococcus faecium* VRE (Van B type), identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin (AMP), imipenem (IPM), and vancomycin. Pink colonies were observed after overnight incubation at 35°C.

- *Enterococcus faecium* VRE (Van A type), identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to teicoplanin and vancomycin. Pink colonies were observed after overnight incubation at 35°C.
- *Enterococcus gallinarum* (Van C type), characterized by CHU Liège using MALDI-TOF and VITEK, showing resistance to vancomycin. Blue colonies or absence of growth were observed after overnight incubation at 35°C.
- *Enterococcus faecalis* ATCC 29212 (Andrewes and Horder) Schleifer and Kilpper-Bal, a reference strain frequently used in antibiotic susceptibility testing. No growth was observed after overnight incubation at 35°C.

ESBL medium: *Escherichia coli* ESBL positive, identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate (AMC), cefuroxime (CRO), cefotaxime (CTX), ceftazidime (CAZ), cefepime (FEP), and ciprofloxacin (CIP). Pink colonies were observed after overnight incubation at 35°C.

- *Klebsiella pneumoniae* ESBL positive, identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, cefepime, ciprofloxacin, and trimethoprim/sulfamethoxazole (TMP-SULFA), and intermediate resistance to nitrofurantoin. Blue colonies were observed after overnight incubation at 35°C.
- *Enterobacter cloacae* complex AmpC (cyclic adenosine monophosphate), identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, cefepime, gentamicin (GEN), and ciprofloxacin, and intermediate resistance to nitrofurantoin. Blue colonies were observed after overnight incubation at 35°C.
- *Pseudomonas aeruginosa* resistant to CAZ, identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, and cefuroxime. Blue colonies were observed after overnight incubation at 35°C.

mSuperCARBA media: *Escherichia coli* VIM (Verona integrin-encoded metallo-beta-lactamase), identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, meropenem (MEM), and trimethoprim/sulfamethoxazole. Early-appearing pink colonies were observed after overnight incubation at 35°C.

- *Klebsiella pneumoniae* carbapenemase (KPC), identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, cefepime, meropenem, amikacin (AMK), gentamicin, ciprofloxacin, nitrofurantoin (NFT), fosfomycin (FO), and trimethoprim/sulfamethoxazole. Blue colonies were observed after overnight incubation at 35°C.
- *Klebsiella pneumonia oxacillinase*-48 (OXA-48), identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, ciprofloxacin, and intermediate resistance to nitrofurantoin. Blue colonies were observed after overnight incubation at 35°C.
- *Enterobacter cloacae* complex VIM, identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, cefepime, meropenem, gentamicin, fosfomycin, and trimethoprim/sulfamethoxazole, and intermediate resistance to amikacin. Blue colonies were observed after overnight incubation at 35°C.
- Carbapenem-resistant *Pseudomonas aeruginosa*, identified by CHU Liège using MALDI-TOF and VITEK, showing resistance to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, and cefuroxime. Cream-colored or transparent colonies were observed after overnight incubation at 35°C.
- *Escherichia coli* ATCC 25922 (Migula) Castellani and Chalmers (Organisation mondiale de la Santé, 2013), a reference strain widely used for antibiotic susceptibility testing. No growth was observed after overnight incubation at 35°C [17].

Preparation of the media

The preparation of the four selective chromogenic media, namely CHROMagar MRSA, VRE, mSuperCARBA, and ESBL, was carried out manually. To prevent any risk of overflow during sterilization, a 300 milliliter (mL) volume (approximately 15 Petri dishes) was prepared in 500 mL borosilicate flasks (VWR 3.3, reference: 215-1594). This process was repeated three times to produce the three batches required for the study [18-21].

Preparation of MRSA medium: To prepare the powdered base (B) of the MRSA medium (reference: MR500, lot P001533), 82.5 g was carefully dissolved in 1L of purified water until a homogeneous mixture was achieved. This solution was then autoclaved in a Tuttnauer 3850 EL-D autoclave using moist heat at 110°C for 5 minutes at a pressure of 100 kilopascal (kPa). For reconstitution, the powdered supplement (S) (reference: SU620, lot 1902) was

sterilely rehydrated with 20.0 mL of sterile water and then gradually added to the base (B) once it had cooled to 45°C.

Preparation of VRE medium: The powdered base (B) of the VRE medium (reference: VR952, lot P001465) was prepared by carefully dispersing 67.3 gram (g) of the base in 1L of purified water until fully homogenized. This mixture was then autoclaved under the same conditions as the MRSA medium, at 110°C for 5 minutes at a pressure of 100 kPa. To prepare a stock solution of the powdered VRE supplement (S) (reference: VR952, lot P001516), 60 mg of the supplement was dissolved in 1 mL of a solution consisting of 0.5 mL ethanol and 0.5 mL of sterile purified water, stirring until fully dissolved. Once the base (B) had cooled to 45°C, 1 mL of the supplement solution was added and mixed thoroughly until fully incorporated.

Preparation of ESBL medium: In preparing the base (B) of the ESBL medium (reference: RT412, lot P001593), 33 g of powder were gently dispersed in 1L of purified water. The mixture was then heated to boiling (100°C) with slow, consistent stirring. After boiling, it was autoclaved at 121°C for 15 minutes under a pressure of 100 kPa. To prepare the supplement (S), 570 milligrams (mg) of powdered supplement (reference: ES372, lot P001541) were weighed and dissolved in 10 mL of sterile purified water. The supplement solution (S) was homogenized by vortexing, then added to the base (B) after the base had cooled to 45°C.

Preparation of mSuperCARBA medium: To prepare the mSuperCARBA medium, 42.5 g of the powdered base (B) SC172 (lot P001519) was carefully dispersed in 1L of purified water, followed by the addition of 2 mL of liquid supplement (S1) SC172 (lot 171210) to the base. The mixture was then brought to a boil (100°C) without autoclaving. The temperature was monitored using a partial immersion thermometer (reference: 11561993). The mSuperCARBA powdered supplement (S2) (reference: SC172, lot P001602) was reconstituted by dissolving 250 mg in 2 mL of purified water, stirred until fully dissolved, and then sterilized by 0.45 µm filtration. After cooling the (B and S1) mixture to 45°C, 2 mL of the supplement (S2) was added to complete the preparation.

Following preparation, the media were poured in 20 mL portions into sterile Petri dishes (reference: 101VR20) and left at room temperature on the workbench overnight for solidification and drying.

Storage conditions

The stability of the media was evaluated over a period of 7 days considering various parameters:

- Condition 1: Storage in a refrigerator at a temperature of 2-8°C in darkness, in accordance with the manufacturer's recommendations.
- Condition 2: Storage at 20°C in darkness, a precarious condition.
- Condition 3: Storage at 20°C in light, a precarious condition.
- Condition 4: Storage at 35°C in darkness, a precarious condition.

Quality control (QC) of media

Each batch of prepared medium (MRSA, VRE, mSuperCARBA, ESBL) underwent quality control including tests for sterility, appearance, pH, growth, and selectivity [22].

- **Sterility:** The media were tested over a 7-day period for each condition. Seven Petri dishes containing the medium were incubated without bacterial inoculation, one per day, and sterility was assessed after overnight incubation at 35°C.
- **Appearance:** The appearance of the media was visually evaluated for each batch by observing dryness, surface uniformity, and normal colour of the medium.
- **pH:** The hydrogen potential (pH) of each batch of medium was measured using a (reference: Checker A673.1) pH meter and pH indicator papers.
- **Growth:** The corresponding resistant bacterial strain for each type of medium was inoculated to assess growth. Results were read after overnight incubation at 35°C.
- **Selectivity:** The control bacterial strain (ATCC), which should not grow, and the resistant bacterial strain, which should have normal growth, were inoculated on each type of medium. Results were interpreted after overnight incubation at 35°C.

Analytical validation

Bacterial growth was evaluated by streaking a 0.5 McFarland bacterial suspension using the quadrant method on the prepared media (CLSI Archived Methods, 2024) [23]. After overnight incubation at 35°C, the media were visually assessed. To verify the accuracy, repeatability, intermediate precision, and robustness of the prepared chromogenic media, the methodology used is as follows:

- **Accuracy:** The accuracy was tested by inoculating known strains onto the three prepared batches. (Agence nationale de sécurité sanitaire alimentaire, 2015) [24].
- **Repeatability:** Repeatability was assessed by inoculating known strains in triplicate onto media from the same batch.
- Intermediate Precision or Intra-Laboratory Reproducibility: Three media from the same batch were inoculated over three successive days to assess intermediate precision or intra-laboratory reproducibility.
- **Robustness:** To assess robustness, three operators inoculated three media from the same series with known strains on the same day. For seven days, the media from this series (same batch) stored under different conditions were successively inoculated with the same known strains.

Results

The results obtained in this study are as follows:

Quality control (QC) of media

In the QC phase, 100% satisfactory results were obtained in terms of sterility, growth, selectivity, appearance, and pH.

- **Sterility:** None of the media stored for 1 to 7 days under the manufacturer's recommended conditions showed microbial contamination after overnight incubation at 35°C.
- **Growth:** For each evaluated medium, cultures of the target bacterial species showed growth of colonies with the expected characteristics on these different media after overnight incubation at 35°C.
- **Selectivity:** Control strains, including both sensitive and resistant strains, were correctly differentiated on each type of medium, with inhibition of sensitive strains and growth of resistant strains, as expected after overnight incubation at 35°C.
- **Appearance:** The media maintained a homogeneous appearance, without discoloration or precipitation, throughout the evaluation period.
- **pH:** The pH values of the media remained within the limits specified by the manufacturer (pH between 6 and 7), thus ensuring optimal culture conditions for the tested bacteria.

In the "challenge" tests under storage conditions deviating from recommendations, the QC results of the four prepared media remained stable. The conditions tested included: 1) Storage at 2-8°C in the dark (compliant with recommendations), 2) Storage at 20°C in the dark (precarious), 3) Storage at 20°C in the light (precarious), and 4) Storage at 35°C in the dark (precarious) (Figure 1).

Analytical validation

For each of the four media, the expected results were obtained for accuracy across the three prepared batches, for repeatability across the three series of the same batches, and for intermediate precision over three successive days by the same technician. Regarding robustness, the first part involved inoculation by three agents on the same day, and the expected results were obtained. In the second part, which involved testing the three conditions (temperature, light exposure, and storage duration), the results obtained are presented in tables 1 to 3.

Discussion

At the end of this study, the quality control of the four CHROMagar media proved excellent when prepared according to the manufacturer's, thereby ensuring high-quality media. However, during the validation over a seven-day period under various operational conditions, only the mSuperCARBA and ESBL media maintained satisfactory performance. The MRSA and VRE media revealed limitations related to the conditions and duration of storage.

The accuracy results met expectations for each selective chromogenic medium, with consistent outcomes across three media preparation batches. Repeatability and intermediate precision were also confirmed, with no significant variation observed based on the operator or culture readings. For the MRSA medium, difficulties were identified during preparation, particularly due to autoclaving parameters. For use in rural areas, a potential solution could be to adopt a preparation method similar to that of mSuperCARBA, which involves boiling the medium at 100°C without autoclaving. Additionally, specific storage limitations were observed, with a loss of characteristics beyond certain periods and

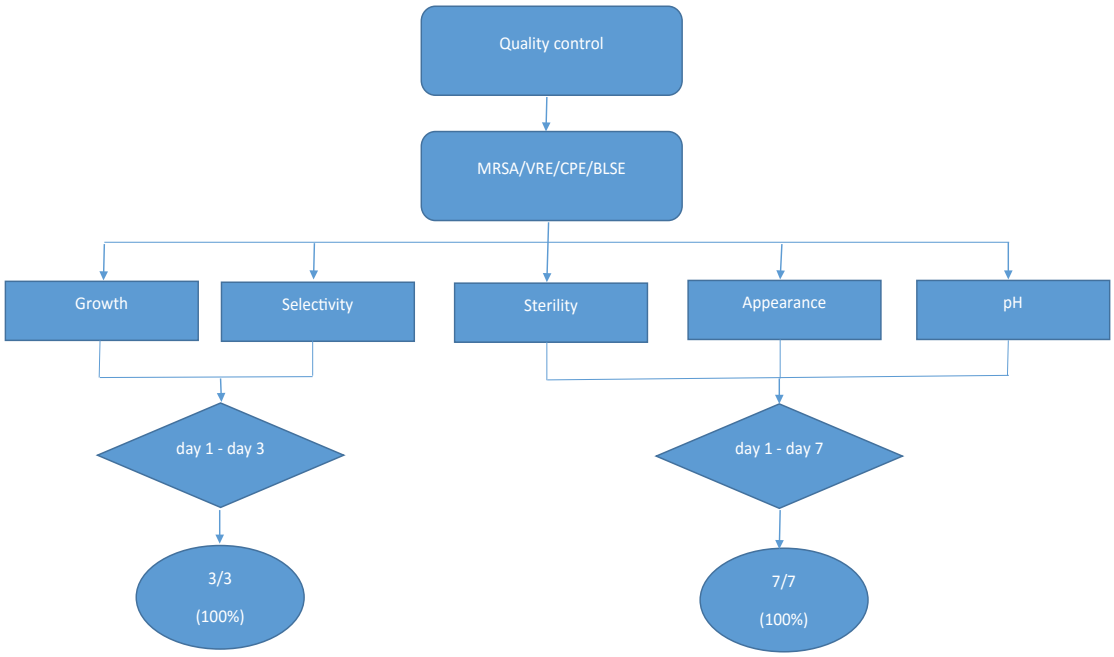

Figure 1: Summary diagram of the quality control of the four media prepared and stored according to recommended conditions, showing the percentage of valid tests per day.

Staphylococcus strain	Expected result after overnight incubation at 35°C	Storage conditions of media, cultivation for 1 to 7 days under defined conditions, observations of colony growth and coloration							
		2-8°C darkness		20°C light		20°C darkness		35°C darkness	
		Growth	Colour	Growth	Colour	Growth	Colour	Growth	Colour
Staphylococcus aureus (MRSA)	pink	7/7(100%)	7/7pink (100%)	7/7(100%)	7/7pink (100%)	7/7(100%)	7/7pink(100%)	7/7(100%)	7/7 pink (100%)
*Staphylococcus epidermidis**	no growth	3/7 (43%)	3/7 pink (43%)	5/7 (71%)	5/7 pink (71%)	4/7 (57%)	4/7 pink (57%)	4/7 (57%)	4/7 pink (57%)
Staphylococcus aureus (MSSA) ATCC 29213	no growth	/	/	/	/	/	/	/	/
*No information from the manufacturer on the expected result for *Staphylococcus epidermidis*.									

1a: CHROMagar MRSA Medium.

Enterococcus strain	Expected result after overnight incubation at 35°C	Storage conditions, culture for 1 to 7 days under defined conditions, observations of colony growth and coloration							
		2-8°C darkness		20°C light		20°C darkness		35°C darkness	
		Growth	Colour	Growth	Colour	Growth	Colour	Growth	Colour
Enterococcus faecium VRE (Van B type)	pink	7/7 (100%)	7/7pink (100%)	5/7 (71%)	5/7 pink (71%)	7/7 (100%)	7/7 pink (100%)	7/7 (100%)	7/7 pink(100%)
Enterococcus faecium VRE (Van A type)	pink	7/7 (100%)	7/7 pink(100%)	5/7 (71%)	5/7 pink (71%)	7/7 (100%)	7/7 pink (100%)	7/7 (100%)	7/7pink (100%)
Enterococcus gallinarum (Van C type)	blue or no growth	/	/	/	/	/	/	/	/
Enterococcus faecalis ATCC 29212	no growth	/	/	/	/	/	/	/	/

1b: CHROMagar VRE Medium.

Enterobacteriaceae strain	Expected result after overnight incubation at 35°C	Storage conditions, culture for 1 to 7 days under defined conditions, observations of colony growth and coloration							
		2-8°C darkness		20°C light		20°C darkness		35°C darkness	
		Growth	Colour	Growth	Colour	Growth	Colour	Growth	Colour
Escherichia coli ESBL	pink	7/7(100%)	7/7pink (100%)	7/7(100%)	7/7 pink(100%)	7/7 (100%)	7/7pink (100%)	7/7 (100%)	7/7 pink(100%)
Klebsiella pneumoniae ESBL	blue	7/7(100%)	7/7 blue(100%)	7/7(100%)	7/7 blue(100%)	7/7 (100%)	7/7 blue (100%)	7/7 (100%)	7/7 blue(100%)
Enterobacter cloacae AmpC complex	blue	7/7(100%)	7/7blue (100%)	7/7(100%)	7/7 blue(100%)	7/7 (100%)	7/7 blue (100%)	7/7 (100%)	7/7 blue(100%)
Pseudomonas aeruginosa R at CAZ	green	7/7(100%)	7/7green(100%)	7/7(100%)	7/7green(100%)	7/7 (100%)	7/7 green(100%)	7/7 (100%)	7/7green(100%)
Escherichia coli ATCC 25922	no growth	/	/	/	/	/	/	/	/

1c: CHROMagar ESBL Medium.

Enterobacteriaceae strain	Expected result after overnight incubation at 35°C	Storage conditions, culturefor 1 to 7 days under defined conditions, observations of colony growth and coloration							
		2-8°C darkness		20°C light		20°C darkness		35°C darkness	
		Growth	Colour	Growth	Colour	Growth	Colour	Growth	Colour
Escherichia coli VIM	pink	7/7(100%)	7/7 pink (100%)	7/7 (100%)	7/7 pink (100%)	7/7 (100%)	7/7 pink(100%)	7/7 (100%)	7/7 pink (100%)
Klebsiella pneumoniae KPC	blue	7/7(100%)	7/7 blue (100%)	7/7 (100%)	7/7 blue (100%)	7/7 (100%)	7/7 blue(100%)	7/7 (100%)	7/7 blue (100%)
Klebsiella pneumoniae OXA-48	no growth	/	/	/	/	/	/	/	/
Enterobacter cloacae complex VIM	blue	7/7(100%)	7/7blue (100%)	7/7 (100%)	7/7blue (100%)	7/7 (100%)	7/7blue (100%)	7/7 (100%)	7/7blue (100%)
Pseudomonas aeruginosa carba R	no growth	/	/	/	/	/	/	/	/
Escherichia coli ATCC 25922	no growth	/	/	/	/	/	/	/	/
Legend: oxacillinases (OXA), American Type Culture Collection (ATCC), cyclic adenosine monophosphate (cAMP), ceftazidime (CAZ), metallo-enzymes (VIM, IMP), carbapenemase (CARBA).									

1d: CHROMagar mSuperCARBA Medium.

Table 1: Results of Inoculation of Strains on the Four Types of Media Prepared Manually and Stored Under Different Conditions.

MRSA	20°C darkness (N=21)	20°C light (N=21)	35°C darkness (N=21)	2/8°C darkness (N=21)
Se	100%	100%	100%	100%
Sp	71%	64%	21%	79%
PPV	64%	58%	39%	70%
PPN	100%	100%	100%	100%

2a: Performance of CHROMagar MRSA Medium.

VRE	20°C darkness (N=28)	20°C light (N=28)	35°C darkness (N=28)	2/8°C darkness (N=28)
Se	100%	78%	100%	100%
Sp	100%	100%	100%	100%
PPV	100%	100%	100%	100%
PPN	100%	71%	100%	100%

2b: Performance of CHROMagar VRE Medium.

ESBL	20°C darkness (N=35)	20°C light (N=35)	35°C darkness (N=35)	2/8°C darkness (N=35)
Se	100%	100%	100%	100%
Sp	100%	100%	100%	100%
PPV	100%	100%	100%	100%
PPN	100%	100%	100%	100%

2c: Performance of CHROMagar ESBL Medium.

CPE	20°C darkness (N=42)	20°C light (N=42)	35°C darkness (N=42)	2/8°C darkness (N=42)
Se	100%	100%	100%	100%
Sp	100%	100%	100%	100%
PPV	100%	100%	100%	100%
PPN	100%	100%	100%	100%
N = number of culture media.				

2d: Performance of CHROMagar mSuperCARBA Medium.

Table 2: Results of Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) Under Different Storage Conditions for the Four Media Prepared Manually, Cultured After 1 to 7 Days According to Defined Conditions.

Medium Type	Storage Duration (days)	Temperature	Light Condition	Sensitivity (Se)	Specificity (Sp)
VRE	1 to 7	20°C	Darkness	100%	100%
	1 to 7	35°C	Darkness	100%	100%
	1 to 7	2-8°C	Darkness	100%	100%
ESBL	1 to 7	20°C	Darkness	100%	100%
	1 to 7	20°C	Light	100%	100%
	1 to 7	35°C	Darkness	100%	100%
	1 to 7	2-8°C	Darkness	100%	100%
CPE	1 to 7	20°C	Darkness	100%	100%
	1 to 7	20°C	Light	100%	100%
	1 to 7	35°C	Darkness	100%	100%
	1 to 7	2-8°C	Darkness	100%	100%

Table 3: Optimal conditions to ensure good sensitivity and specificity under the different tested conditions.
The MRSA medium does not exhibit optimal conditions for sensitivity and specificity (< 80%).

storage conditions. In the literature, BBL CHROMagar™ MRSA medium showed a very high sensitivity of 97.6% [121/124] at 18-24 hours of incubation and 100% [124/124] at 48 hours, with a specificity of 99.9% for identifying MRSA (Pape et al., 2006). In this study, at 20°C in light, the MRSA medium was preserved for 2 days, and for 3 days at 20°C in the dark and at 35°C in the dark. Recommended storage at 2-8°C, in the dark according to the manufacturer's instructions, maintained the quality of the medium for 4 days. Within these storage limits, the results obtained met expectations. Beyond these limits, growth of methicillin sensitive *S. aureus* (MSSA) and *S. epidermidis* was observed. The MRSA medium did not produce the expected results, showing variation under both the manufacturer's recommended conditions and the tested precarious conditions. However, the MRSA medium demonstrated an excellent sensitivity of 100% [28/28] and a low specificity of 59% [33/56] in identifying MRSA. As noted in the literature, non-MRSA isolates can sometimes grow as slightly pink colonies on the chromogenic medium [25]. In this case, at 35°C in the dark, after 1 day, the results did not meet expectations. The hypothesis is that the high temperature during the night of incubation inhibited the antibiotics in the medium, leading to the growth of sensitive bacterial colonies, such as *S. epidermidis*, or a whitish pink coloration for MSSA ATCC 29213, which showed growth. This would explain the unexpected results from the 5th day in different series, despite the manufacturer's recommendation for storage up to 30 days at 2-8°C. Chromogenic agars produced by other manufacturers have also been evaluated in other studies and have shown to be both sensitive and specific for the detection of MRSA [26,27].

A note regarding the single vial of supplement emphasizes that repeated use could lead to qualitative variations in the content. Repeated use of the lyophilized vial may alter results, whereas freezing in aliquots would avoid these temperature variations.

For the VRE medium, results met expectations under the manufacturer's recommended conditions, but growth issues were observed after a certain storage duration in light, suggesting a link with this environmental variable. Light could be the cause of enzymatic inhibition preventing the growth of resistant bacteria. This medium showed a performance of 100% specificity [52/52] and 93% sensitivity [56/60]. Similar results were obtained in previous studies evaluating the sensitivity of chromogenic VRE media [28,29]. Regarding specificity, previous studies had obtained values higher than

95%. However, some studies showed a sensitivity of 96% but a low specificity of 30% [30].

Accuracy, repeatability, intermediate precision, and robustness for the mSuperCARBA and ESBL media showed results consistent with expectations. All strains exhibited the expected characteristics, and no variation in results was observed based on condition 1 recommended by the manufacturer. No storage limits were detected during tests over a 7-day period. One study reported a sensitivity of 98% and a specificity of 89% for the ESBL chromogenic medium [31]. In this study, the ESBL medium demonstrated a sensitivity and specificity of 100% [140/140] for *E. coli* and KEC (*Klebsiella, Enterobacter, Citrobacter*). Similarly, the mSuperCARBA medium also showed a sensitivity and specificity of 100% [160/160] for *E. coli* and KEC. These results are in line with the literature, which reports a sensitivity of 100% for detecting KPC non-OXA-48 and a specificity of 99% for detecting nonKPC enterobacteria on the CARBA chromogenic medium [32]. Furthermore, the mSuperCARBA medium showed the highest sensitivity with both low and high inoculum [33].

This study demonstrates that after 7 days of storage under optimal conditions, the 4 media maintained expected appearance and sterility, as well as a stable pH of 7. Robustness was confirmed for each chromogenic medium. These media allow for rapid presumptive identification of the targeted pathogens, thereby underscoring their critical importance to public health. Due to their ease of preparation and storage, these media can serve as a key component in the first line of defense for monitoring antimicrobial resistance (AMR). They enable local health centres to carry out the initial steps in bacterial identification (steps 1 and 2), thus facilitating the subsequent transfer of samples for antimicrobial susceptibility testing (AST), which would be performed at regional hospitals or public health institutes. This provides clinicians with an early indication of the bacterial genus, and potentially the species and resistance patterns, allowing for quicker and more appropriate therapeutic adjustments.

Moreover, the collection of samples from community health centres would offer a more representative view of the local AMR landscape, contributing to the decentralization of antimicrobial resistance data collection. Currently, public health reference centres, responsible for surveillance, only receive samples from regional hospitals, which limits the data to a centralized approach. Patients from community health centres, even in cases of visible sepsis (pus, wounds, etc.), often avoid regional hospitals and opt for empirical antibiotic prescriptions. This frequently leads physicians to prescribe third- or fourth-generation antibiotics, as follow-up visits by these patients are rare.

However, there are human and technical limitations. The interpretation of results can be affected by lighting conditions, although this issue can be mitigated by ensuring consistent reading in a stable environment. Additionally, non-resistant colonies can sometimes grow on media designed to detect resistance, requiring increased vigilance. Finally, exposure of the media powders to light can affect the results, highlighting the need for strict storage practices away from light.

Conclusion

Under optimal conditions, the mSuperCARBA, ESBL, VRE, and MRSA media used in this study exhibit excellent sensitivity and good specificity for detecting bacterial resistances. However, in precarious conditions, such as those found in rural areas of low- and middle-income countries, it is essential to carefully monitor the post-preparation storage conditions of the MRSA medium: up to 2 days at 20°C under light or in darkness, 1 day at 35°C, 3 days at 20°C in darkness, and 4 days at 2-8°C. Similarly, the VRE medium should be stored for a maximum of 5 days at 20°C under light, but can last up to 7 days when stored at 35°C, 20°C, or 2-8°C in darkness. In contrast, the mSuperCARBA and ESBL media remain stable under all tested storage conditions (2-8°C, 20°C, 35°C) in both light and dark. These results are thus reproducible and reliable.

In conclusion, this study demonstrates the feasibility of using CHROMagar chromogenic media to detect resistant bacteria such as MRSA, VRE, ESBL, and KPC, with sensitivities ranging from 93% to 100% and specificities from 59% to 100%. The ease of storage and preparation of these media allows for their routine use in resource-limited countries. Although the MRSA and VRE media are sensitive to temperature and light, a field study is required to confirm these results, along with confirmatory analysis of the identified strains at the CHU de Liège clinical microbiology laboratory. These media are reliable, easy to prepare, and less time-consuming, making them economically advantageous.

These media present an affordable option for detecting MRSA, VRE, ESBL, and KPC in low- and middle-income countries, which is critical for effective infection management and improved therapeutic monitoring of patients. Their use, both in routine diagnostics and surveillance, is crucial for limiting the spread of infections and ensuring optimal treatment. Additionally, chromogenic media would facilitate the decentralization of the initial steps of bacterial identification, contributing to a more efficient surveillance of antimicrobial resistance (AMR), which is currently centralized in reference laboratories.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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