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Comparative Evaluation of Two Commercial Chromogenic Media for Detection and Presumptive Identification of Urinary Tract Pathogens

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Abstract The performance of two commercial chromogenic media for the isolation and presumptive identification of urinary tract pathogens, the CPS ID2 (bioMérieux, France) and the CHROMagar Orientation (BBL Becton Dickinson, USA), was evaluated and compared with that of cystine-lactose-electrolyte-deficient agar and tryptic soy agar with 5% sheep blood. The detection, determination of bacterial counts, and presumptive identification of bacteria causing urinary tract infections were evaluated in 3,000 urine specimens. The two chromogenic media showed excellent correlation with the standard media for the detection and the bacterial count of urinary pathogens. The *Escherichia coli* strains produced the expected colour on the CHROMagar Orientation and the CPS ID2 media in 99% and 90% of the cases, respectively. The *Klebsiella-Enterobacter-Citrobacter* and the *Proteus-Morganella-Providencia* groups were easily identified on both chromogenic media, but further biochemical tests were needed to differentiate them to a species level. Both media enabled the differentiation, with varying degrees of difficulty, of *Pseudomonas* spp. strains from members of the family *Enterobacteriaceae*. All isolates of *Enterococcus* spp. were correctly identified and were easily distinguished from the *Streptococcus agalactiae* isolates. *Staphylococcus saprophyticus* isolates were easy to identify only on the CHROMagar Orientation medium. No substantial difference was observed when comparing the results of the susceptibility tests, which were performed according to the standardized disk diffusion method as described by the National Committee for Clinical Laboratory Standards, for colonies recovered from the blood agar versus those recovered from the chromogenic media. In conclusion, the CPS ID2 and CHROMagar Orientation media enabled excellent detection, count determination, and presumptive identification of urinary pathogens, both in pure and mixed cultures, and reliable and accurate antimicrobial susceptibility testing directly from primary isolates. Moreover, these media allowed a remarkable reduction in the workload and a significant savings of time. On the basis of their performance, these media can replace the standard primary plating media used in the routine diagnosis of urinary tract infections.

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Introduction

The microbiology laboratory carries out a fundamental role in the diagnosis, therapy, and monitoring of urinary tract infections. A rapid identification of the etiological agent can provide the clinician with precious information regarding the appropriate choice of an antibiotic, even before the results of the susceptibility test are available [1]. Due to the high incidence of urinary tract infections, the number of relevant microbiological tests that are required account for a considerable workload in the daily routine of the laboratory. Culture is the testing method for screening urine samples, and the reference standard media include tryptic soy agar (TSA) with 5% sheep blood, MacConkey agar, and Columbia colistine-nalidixic acid agar [2, 3].

New chromogenic media have been developed in order to facilitate and speed up the presumptive identification of the main bacteria isolated from urinary samples [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. The presumptive identification is based on the different colourings of the bacterial colonies following the reactions between species-specific or genus-specific enzymes and the chromogenic substrates incorporated in the medium; only a few additional confirmatory tests are required.

Most patients with urinary tract infections need to be treated before the results of the susceptibility test are available. The choice of the appropriate drug depends on the identification and susceptibility of the microorganism. In order to perform the disk diffusion antibacterial susceptibility test as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), colonies isolated from a nonselective medium, such as blood

agar, must be utilized to prepare the suspension to be inoculated onto the Mueller-Hinton agar [16].

The objective of this study was to evaluate CPS ID2 (CPS) medium (bioMérieux, France) and BBL CHROMagar Orientation (CHR) medium (BBL Becton Dickinson, USA) for the detection, count determination, and presumptive identification of the bacteria responsible for urinary tract infections and compare them with two traditional control media: a cystine-lactose-electrolyte-deficient (CLED) medium and a tryptic soy agar (TSA) with 5% sheep blood. Additionally, the susceptibility tests performed on the colonies isolated from the two chromogenic media and the control medium (TSA with 5% sheep blood) were evaluated and compared. In order to standardize the readings of the zones of inhibition, we utilized a BioVideobact computerized instrument (Biokit, Spain) equipped with a video camera.

Materials and Methods

Urine Samples

Culture screening tests for the detection of bacteriuria were carried out on 3,000 random urine samples received consecutively at our microbiology laboratory. A total of 1,217 specimens were collected from outpatients, and 1,783 specimens were collected from patients hospitalized in different departments of our hospital as follows: 253 from the nephrology and the kidney transplant units, 202 from the haematology ward, 171 from the geriatric ward, 165 from the metabolic diseases department, 156 from the paediatric ward, 141 from the obstetrics and gynaecology ward, 139 from the urology ward, 123 from the internal medicine department, 102 from the surgery department, 97 from the intensive care unit, and 234 from other miscellaneous departments. Most of the urine specimens (2,710 from clean-catch midstream urine and 243 from indwelling bladder catheters) were collected in sterile plastic containers, while 57 urine specimens, the ones from paediatric patients, were collected with the application of a sterile plastic adhesive bag. All the samples were processed within 2 h after collection or were forwarded to the laboratory in refrigerated conditions, stored at 4°C, and processed within 4 h after collection.

Detection of Antimicrobial Agents

To detect the presence of antimicrobial agents in each urine sample, antimicrobial test plates were performed. A 6 mm diameter paper disk was dipped into the urine sample and placed onto a Mueller-Hinton agar plate seeded with a fully sensitive strain of *Staphylococcus epidermidis* (ATCC 12228). Plates were incubated at 35–37°C in aerobic conditions and examined after 18–24 h for zones of inhibition around the disk. Antimicrobial substances were detected in 405 urine specimens. Of these specimens, 203 yielded clinically significant bacteriuria and 202 yielded nonsignificant bacteriuria or no growth.

Microscopy

For each of the urine samples, a microscopic examination and cell count were performed on well-mixed uncentrifuged urine samples by using Kova slides with 10 counting grids. Each of the 10 chambers holds a standardized 6.6 µl volume of the sample. A sample was considered positive for the presence of pyuria when the count was ≥10 leucocytes/µl. Squamous epithelial cells were recorded as "few" (<5 cells per low-power field), "moderate" (6–15 cells per low-power field), or "numerous" (>15 cells per low-power field). The presence of bacteria was also recorded. Microorganisms were classified for morphology, and the approximate number per high-power field was quantified.

Test Media Used and Inoculation Technique

The CPS medium (bioMérieux, France) and the CHR medium (Becton Dickinson, USA) are sold as prepared plated media. The TSA medium (Difco Laboratories, USA) and the CLED medium (Becton Dickinson, USA) were prepared from dehydrated powder, according to the instructions provided by the manufacturer. For the preparation of blood plates, 5% sterile defibrinated sheep blood was added to sterile agar that previously had been melted and cooled to 45–50°C. The media were dispensed in 90 mm diameter sterile petri capsules, which were stored at 4°C and utilized within 4 weeks. Urine samples were directly inoculated in parallel conditions on four media with a 0.0075 ml calibrated loop by means of a semiautomatic Urine Robot (p.b.i. International, Italy). For determination of the bacterial count, the volume of the "nonstandard" calibrated loop supplied with the Urine Robot was converted to 0.01 ml using a conversion factor (1 colony equals 133 colony-forming units [cfu]/ml). This system was used in order to guarantee standardization of the inoculating process; the technique employed was the standard surface-streak method. The plates were then incubated at 37°C for 18–20 h [2, 3].

Quality Control

Each batch of medium prepared in the laboratory, or a new lot of the two commercial media, was tested for sterility, ability to support the growth, and chromogenic and biochemical response with American Type Culture Collection (ATCC) control strains. The *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 strains were inoculated in brain heart infusion broth (BBL Becton Dickinson) and incubated for 4–5 h. After adjusting the turbidity level to the standard of 0.5 McFarland, the suspensions, previously diluted 1:100 in physiological solution, were inoculated on the surface of the media by means of the Urine Robot calibrated loop.

Interpretation of Results

Guidelines for processing urine samples in our laboratory have been established based on Cumitech 2B [2] and on a close cooperation between the clinical and laboratory staff. The following criteria were adopted for the microbiological assessment of urine specimens: (i) a colony count of $\geq 10^5$ cfu/ml of one or two species of probable pathogens, with the possible presence of other isolates in low concentrations ($\leq 10^4$) that were ignored, as the criterion for diagnosis of pyelonephritis, acute cystitis, and asymptomatic bacteriuria; (ii) a colony count of 10^2–10^5 cfu/ml of one or two species of probable pathogens from symptomatic patients (women with frequency-dysuria syndrome, symptomatic males, and selected urology, paediatric, and geriatric patients), from patients with indwelling bladder catheters, from urine specimens collected by in-and-out (straight) catheterization or suprapubic aspiration and from other specimens for which special cultures are recommended [2].

Patients with complicated infections (associated with stones, renal abscesses, or long-indwelling urinary catheters) often will have multiple organisms in their urine. As many as four to five or more different species may be recovered from patients on long-term catheter drainage, and the clinical microbiology laboratory should be alerted to the possibility of polymicrobic bacteriuria to avoid rejecting the specimen as contaminated. Nevertheless, when possible, it is preferable to collect urine specimens after placement of a new catheter rather than from a catheter that has been in place for an extended time, because the latter often develops a microbial flora different from that present within the patient's urinary tract, in which case urine specimens from the collecting tubing may not reflect the microbiological status of the patient's urinary tract [2].

Detection of a bacterial count of $<10^4$ cfu/ml in nonselected patients devoid of clinical information was interpreted as nonsignificant bacteriuria; detection of sterile cultures was evaluated as no growth. Both of these types of cultures were considered negative. Detection of more than two species of bacteria in low concentrations from urine specimens was interpreted as a contaminated (mixed) culture.

The counts obtained from the analysis of the two chromogenic media were compared with those obtained with the CLED medium. CLED is a differential, nonselective medium that favours the growth of most of the pathogenic urinary bacteria. It provides sufficient differentiation between the colonies and enables the detection of lactose-fermenting gram-negative enteric bacteria. It inhibits the swarming of *Proteus* and other motile bacteria. TSA with 5% sheep blood was used predominantly for the detection of urinary pathogens, in particular the more fastidious bacteria, but not for determining the count because it has several disadvantages, such as the minimal differentiation between the organisms recovered and its complete failure to inhibit the swarming of *Proteus* and other motile bacteria.

Presumptive Identification of Bacteria

CPS and CHR are culture media containing specific chromogenic substrates for particular enzymes such as β-glucuronidase on CPS medium and β-galactosidase on CHR medium, which indicate the development of colonies that are coloured from pink to bordeaux, and β-glucosidase enzyme, which indicates the development of colonies coloured from blue to greenish-blue on both media. With these two media, it is also possible to utilize two supplementary reagents in order to detect two other enzyme-related activities: tryptophanase (indole test) and tryptophan deaminase. The activity of the tryptophan deaminase enzyme is characteristic for the *Proteus-Morganella-Providencia* group and, when this activity ranges from medium to strong, there is a spontaneous and clearly visible production of a diffuse brown pigment that colours the medium around the colonies on both media, even without utilizing a specific reagent ($FeCl_3$). According to the manufacturer's instructions, the media enable the identification of the most common microorganisms involved in urinary tract infections as follows: (i) *Escherichia coli* isolates produce a pink to bordeaux colony colour; a confirmatory test for a definitive identification must be performed by detection of indole production (indole test); (ii) *Enterococcus* spp. isolates produce small colonies with blue to turquoise colour, and a microscopic examination is required to confirm the morphology of the organisms (cocci in chains). On CPS medium, the colony colour and the microscopic evaluation allow the identification of *Enterococcus* spp., while on CHR medium, identification is confirmed by an L-pyrrolidonyl-β-naphthalamide hydrolysis test; (iii) *Proteus mirabilis* isolates produce colourless or beige colony colour, surrounded by a brown halo, and a confirmatory test for a definitive identification must be performed by detection of indole production using a drop of Kovac's reagent. *Proteus penneri* isolates are indistinguishable from *Proteus mirabilis* isolates (both indole negative) on chromogenic media. Nevertheless, *Proteus penneri* strains are occasionally isolated from urine specimens, and they are chloramphenicol resistant and can be easily distinguished from *Proteus mirabilis* strains by an antimicrobial susceptibility test.

The two media also enable the presumptive identification of the following: (i) the *Klebsiella-Enterobacter-Serratia* group, which develop colonies that are coloured from metallic blue to blue-green; a microscopic evaluation is required to confirm morphology (bacilli); and (ii) the *Morganella-Proteus* indole-positive-*Providencia* group, whose colonies are colourless, beige, or blue-green, surrounded by a brown halo, and produce a positive indole reaction. The latter two groups of bacteria, however, require further biochemical tests for correct identification of the species.

CHR medium is also claimed to allow the presumptive identification of *Staphylococcus saprophyticus*, on the basis of the light-pink colour of the colonies and a microscopic examination, which is required to confirm the morphology (cocci).

The indole test on CPS (ID indole; bioMérieux) and on CHR (DMACA Indole Dropper reagent; BBL Becton Dickinson) can be carried out on filter paper. Further confirmatory tests, such as catalase (3% H_2O_2), the Slide Staph-Kit (bioMérieux), the Streptex acid extraction kit (Murex Diagnostics, France), and the oxidase (N, N, N′, N′-tetramethyl-p-phenylendiamine dihydrochloride; Carlo Erba, Italy) tests were carried out according to the manufacturer's instructions. In all cases of dubious results, or whenever further confirmatory identification was required, system identification profiles were used as follows: ID 32 E for *Enterobacteriaceae*; API 20 NE for nonfermenting gram-negative rods; ID 32 Staph for staphylococci; API 20 Strep for streptococci; and ID 32 C for the yeasts (bioMérieux).

Antimicrobial Susceptibility Testing

A performance standard for antimicrobial disk susceptibility testing was carried out on 200 gram-negative isolates and 100 gram-positive isolates, taking samples of the colonies directly from the blood agar according to the NCCLS guidelines [16]. Antimicrobial susceptibility tests of the same bacteria were prepared concurrently by taking colonies from the CHR medium and from the CPS medium. The results of the three tests were evaluated by means of computerized readings of the three corresponding plates, using the instrument BioVideobact (Biokit), which guarantees standardized readings of the zones of inhibition that are produced on the surface of the Mueller-Hinton medium. The readings were evaluated in accordance with the guidelines indicated in the NCCLS documents M2-A6 [16] and M7-A4 [17]. BioVideobact automatically calculates the size of the zones of inhibition by means of a video camera, determines the category of interpretation, and through the interpolation with a specific regression line, it also calculates the minimum inhibitory concentration.

Results

In this study, a routine examination of 3,000 urine specimens was carried out for microbiological evaluation; 1,694 cultures were devoid of bacterial growth, while 381 cultures developed clinically nonrelevant bacteria ($<10^4$ cfu/ml) or developed more than two species in low concentrations. The remaining 925 (30.8%) urine specimens were considered positive and clinically significant. A single bacterial species was isolated in 829 of these samples, and two bacterial species were isolated in 96 specimens. In all cultures of the latter group, both isolates developed a significant colony count. A total of 1,021 bacterial strains were isolated, 954 with a bacterial count $\geq 10^5$ cfu/ml and 67 with a bacterial count between $\geq 10^2$ and $<10^5$ cfu/ml (Table 1).

The fertility of the media was evaluated on the basis of the plate readings, which were carried out after 18–20 h of incubation. Complete correspondence in the results on both chromogenic media was detected, proving that both media were able to support the growth of all the isolates. Valid results were also obtained with regard to the bacterial count, except for five cultures (3 that contained strains of *Candida* spp., 1 that contained *Enterococcus* spp. growing on CPS medium, and 1 contained *Enterococcus* spp. strain growing on CHR medium), all of which contained bacterial counts of $<10^5$ cfu/ml, while on the corresponding chromogenic medium and on the CLED medium, the bacterial count was $\geq 10^5$ cfu/ml.

Table 1 Number of clinically important isolates recovered from 925 urine samples

Species	Total strains isolated	No. of clinically important strains isolated					
		CHR medium		CPS medium		CLED medium	
		≥10^5 cfu/ml	<10^5 cfu/ml	≥10^5 cfu/ml	<10^5 cfu/ml	≥10^5 cfu/ml	<10^5 cfu/ml
Acinetobacter spp.	2	2	0	2	0	2	0
Candida spp.	31	27	4	24	7	27	4
Citrobacter spp.	16	16	0	16	0	16	0
Escherichia coli[a]	429	392	32	397	32	386	32
Enterobacter spp.	17	17	0	17	0	17	0
Enterococcus spp.	213	201	12	201	12	202	11
Hafnia alvei	3	3	0	3	0	3	0
Klebsiella spp.	96	87	9	87	9	87	9
PMP group[b]	89	87	2	87	2	87	2
Pseudomonas spp.	57	48	9	48	9	48	9
Salmonella spp.	1	1	0	1	0	1	0
Streptococcus agalactiae	36	36	0	36	0	36	0
Serratia marcescens	6	6	0	6	0	6	0
Staphylococcus spp.	25	25	0	25	0	25	0
Total	1,021	948	68	950	71	943	67

[a] Eleven urine samples on CPS medium and five urine samples on CHR medium with a double bacterial isolation contained two *Escherichia coli* variants, but only one strain was identified on CLED medium

[b] *Proteus-Morganella-Providencia* group

Table 2 lists the different bacterial species that were isolated and the colour and morphological characteristics of the corresponding colonies on the two chromogenic media. *Escherichia coli* was the predominant species (429 isolates). On the CHR medium, 425 strains developed colonies with the characteristic pink colour; only four strains failed to produce a chromogenic reaction, showing beige colonies. On the CPS medium, 386 strains developed colonies with the expected pink-to-bordeaux colour, while 43 strains were negative β-glucuronidase, producing colonies from colourless to beige. Of all 429 *Escherichia coli* isolates, 3 exhibited a negative indole reaction, 2 of which produced beige colonies on both chromogenic media.

Eleven cases of double bacterial isolation were found in which the two isolates were identified as two different strains of *Escherichia coli*. These strains were not detected on CLED or TSA with 5% sheep blood, but they were clearly identifiable due to the different colouring characteristics of the colonies on CPS medium (pink to bordeaux or pale pink, colourless, beige) in all 11 cases and on CHR medium (pink or pale pink, beige) in 5 cases. These two variants of *Escherichia coli* showed some differences in susceptibility patterns that resulted in a significant change in both the interpretation of the susceptibility test results (S↔R) and in the standard biochemical profiles used to identify the strains correctly. These differences confirmed the presence of two different *Escherichia coli* strains responsible for urinary tract infections.

All *Enterococcus* spp. isolates (n=213) were correctly detected based on the appearance of typical small blue-to-turquoise colonies (β-glucosidase positive) and by microscopic examination (cocci in chains) on both chromogenic media; no false-negative results were obtained. An additional L-pyrrolidonyl-β-naphthalamide hydrolysis test, performed to confirm precise genus identification on CHR medium, was positive for all 213 strains that yielded small blue-to-turquoise colonies and cocci in chains; no false-positive results were obtained. These media allow easy differentiation of a mixed culture of *Enterococcus* spp. and members of the *Klebsiella-Enterobacter-Serratia* group because of the different colour and size of the colonies. Furthermore, *Enterococcus* spp. isolates are nearly always easy to differentiate from *Streptococcus agalactiae* isolates (n=36). The *Streptococcus agalactiae* isolates produced light-blue pin-like colonies on CHR and white, light-blue-to-turquoise pin-like colonies on CPS medium, except for four strains that produced pink-coloured colonies on the latter medium.

Both chromogenic media performed as well as the standard reference media in supporting the growth of *Staphylococcus* spp. isolates. In this study, the following species were isolated: *Staphylococcus aureus* (n=10), *Staphylococcus epidermidis* (n=4), *Staphylococcus saprophyticus* (n=6), *Staphylococcus simulans* (n=2), *Staphylococcus warneri* (n=2), and *Staphylococcus haemolyticus* (n=1). *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates produced colonies that ranged from yellow-golden to opaque-white colour. *Staphylococcus saprophyticus* isolates produced pink-coloured colonies on CHR medium and white-coloured colonies on CPS medium. *Staphylococcus simulans* isolates produced pink-coloured colonies on CHR medium, which could not be distinguished from colonies of the *Staphylococcus saprophyticus* isolates, while *Staphylococcus warneri* isolates and *Staphylococcus haemolyticus* isolates produced pink-coloured colonies on CPS medium.

The white, creamy, convex colonies of *Candida albicans* were difficult to differentiate from the *Candida* spp. isolates on CHR medium, while on the CPS medi-

Table 2 Colour of colonies on CHR medium and on CPS medium

Organism	Total no. of isolates	CHR medium		CPS medium	
		No. (%) of isolates with described colour	Description of pigment and/or morphology of colonies	No. (%) of isolates with described colour	Description of pigment and/or morphology of colonies
Acinetobacter spp.	2	2 (100)	beige	2 (100)	beige
Candida spp.	31	31 (100)	white, creamy, convex	31 (100)	white, creamy, convex
Citrobacter spp.	16	14 (87.5)	metallic blue with or without pink halo	10 (62.5)	blue-green
		2 (22.5)	pink	6 (37.5)	beige
Escherichia coli	429	425 (99)	pink	386 (90)	pink to bordeaux
		4 (1)	beige	43 (10)	colourless to beige
Enterobacter spp.	17	17 (100)	metallic blue with or without pink halo	17 (100)	blue-green
Enterococcus spp.	213	213 (100)	blue to turquoise, small	213 (100)	blue to turquoise, small
Hafnia alvei	3	2 (66.7)	beige	3 (100)	beige
		1 (33.3)	pink with blue halo		
Klebsiella spp.	96	96 (100)	metallic blue with or without pink halo	96 (100)	blue-green
Morganella morganii	7	7 (100)	colourless to beige with brown halo	7 (100)	colourless to beige with brown halo
Proteus mirabilis	61	61 (100)	beige with brown halo	61 (100)	beige with brown halo
Proteus vulgaris	5	3 (60)	beige with brown halo	5 (100)	beige with brown halo
		2 (40)	blue-green with brown halo		
Providencia spp.	16	16 (100)	beige with brown halo	16 (100)	beige with brown halo
Pseudomonas spp.	57	53 (93)	transparent, yellow to green serrated edge, diffused	35 (61.5)	transparent, yellow to green serrated edge, diffused
		4 (7)	beige with or without green halo	22 (38.5)	beige with or without green halo
Salmonella spp.	1	1 (100)	beige	1 (100)	beige
Streptococcus agalactiae	36	36 (100)	light blue, pin-like	32 (88.9)	white, light blue, pin-like
				4 (11.1)	pink
Serratia marcescens	6	6 (100)	blue-green	3 (50)	blue-green
				3 (50)	beige
Staphylococcus saprophyticus	6	6 (100)	pink opaque	6 (100)	white
Staphylococcus spp.	19	19 (100)	golden opaque, white, pink	19 (100)	golden opaque, white, pink

um, the isolates of the latter group showed poor growth and developed very small colonies that were not always easy to see.

Six *Lactobacillus* spp. isolates produced blue-coloured colonies on CHR medium, with a count $\geq 10^5$ cfu/ml; these colonies did not develop on CPS medium.

The zone diameters in the standardized disk diffusion test, from blood agar and from the two chromogenic media, were measured and the distributions of the differences in the zones of inhibition of each antimicrobial agent were recorded. From the comparison of the results obtained in the susceptibility test carried out by taking samples of the colonies from the three different media, a nearly complete concordance was noted. Two minor errors (I↔R) were noted when comparing the tests carried out on gram-negative bacteria, and two minor errors (I↔R) were likewise noted when comparing the tests carried out on gram-positive bacteria.

Discussion

The conventional method for the microbiological diagnosis of urinary tract infections is the quantitative culture of urine samples on standard solid media. The need to introduce new technological solutions in this method has become indispensable for two reasons. First, only a minor percentage (20–30%) of the large number of urine samples that the microbiology laboratory must analyze every day are infected; thus, a remarkable amount of time and culture media is wasted on the evaluation of samples that are mostly clinically nonsignificant. Secondly, there is a need for rapid preliminary identification of the etiological agent in order to enable the clinician to begin the appropriate therapy before the results of susceptibility testing are available. Both the laboratory results and the therapeutic regimen must therefore be available in a shorter period of time. Undoubtedly, an

important aspect of every study is the proven validity of the method, which is essential in order to maintain high-quality, reliable diagnostics.

In an attempt to meet the above requirements, the performances of commercially available CHR and CPS chromogenic media were evaluated and compared with one another as well as with other standard media employed in the routine work of a clinical microbiology laboratory. Our data showed the excellent ability of both chromogenic media to detect the different species of urinary tract pathogens, to support the growth of all the isolates, and to evaluate their growth quantitatively; up to now, the combined use of two conventional media has been required to achieve detection, growth, and quantitative assessment. Unlike blood agar, these chromogenic media inhibit or significantly decrease the swarming of *Proteus*, *Escherichia coli*, and *Klebsiella* mucosal strains, thus enabling a bacterial count and a correct identification of the colonies to be obtained.

The most common microorganisms that produce urinary tract infections are gram-negative bacteria, which belong to the *Enterobacteriaceae* family. CHR and CPS media used different chromogenic substrates to detect specific bacterial enzymes, which allow identification of *Escherichia coli*. Presumptive identification of *Escherichia coli* strains, which accounted for 42% of the clinically significant isolates in our study, was better on CHR medium (99% identified), which detects β-galactosidase enzyme activity, than on CPS medium (90% identified), which detects β-glucuronidase enzyme activity. This difference is statistically significant ($P<0.001$, chi-square test). The superior sensitivity of β-galactosidase enzyme activity in comparison with β-glucuronidase enzyme activity for identification of *Escherichia coli* has recently been reported [6, 14].

In contrast, a better detection of multiple *Escherichia coli* variants in the same urine specimen was observed on CPS medium when compared to CHR medium. This finding is in accordance with the results of the study of Carricajo et al. [14]. Only the chromogenic media enabled detection of these *Escherichia coli* variants, and a correct differentiation is particularly important in order to establish suitable antibiotic treatment.

On these chromogenic media, a correct identification of all pink-to-bordeaux colonies must be confirmed by an indole test, since some strains of *Salmonella* spp., *Citrobacter freundii*, and *Shigella sonnei* also produce β-glucuronidase or β-galactosidase activity, showing pink-to-bordeaux colonies [6, 8, 9, 10]. However, most of these strains are isolated only occasionally from urine specimens and are indole negative. Nevertheless, some strains of *Citrobacter diversus* may also yield pink-to-red colonies on CHR medium and produce a positive indole test result, leading to misidentification as *Escherichia coli* strains (Table 2).

Enterococci were correctly identified on both chromogenic media at the genus level. The two most common enterococcal species, *Enterococcus faecalis* and *Enterococcus faecium*, showed similar colony colour and were indistinguishable at the species level, which is in accordance with the results of other reports [6, 9]. In our study, enterococci represented the second most common cause of urinary tract infection (20.9%); they also caused nosocomial infections and most often were present in catheter-associated cystitis.

All of the *Proteus mirabilis* isolates were chromogenically distinct on both chromogenic media, which yielded the same rate of detection for all isolates of the *Proteus-Morganella-Providencia* group. However, five of the indole-negative isolates of *Proteus mirabilis* were misidentified as indole producers when these strains were isolated in association with an indole-positive strain of *Escherichia coli* or *Klebsiella pneumoniae* var. *oxytoca*. The possibility that the metabolite of the tryptophanase can spread in the medium was suggested previously [8, 14], and for correct identification, it is recommended that all strains detected in mixed cultures that contain at least one indole-positive isolate be confirmed by conventional biochemical tests.

The *Klebsiella*, *Enterobacter*, and *Citrobacter* isolates were easily identified by the production of large metallic-blue and blue-green colonies on CHR and CPS chromogenic media, but further biochemical tests were needed to differentiate them to a species level.

The *Pseudomonas* spp. strains were differentiated and chromogenically distinguished, with varying degrees of difficulty, from the *Enterobacteriaceae* members on both chromogenic media, but further biochemical tests were needed for a correct identification at the species level. Only the CHR medium enabled correct identification of the *Staphylococcus saprophyticus* isolates, which produced typical pink-coloured colonies, as confirmed by previous reports [6, 11]. In our study, two *Staphylococcus simulans* isolates also produced pink-coloured colonies.

The routine processing of urine samples requires that the laboratory staff be able to evaluate plates with mixed cultures, because often the absence of particular differentiating characteristics will hinder the different bacterial strains from being recognized. The differences in colour and size of the colonies on these two chromogenic media enabled easy differentiation of the isolates in mixed cultures.

The well-distinguished colours produced by some of the species enable rapid presumptive identification of the main bacteria responsible for urinary tract infections; only a few additional confirmatory tests are required. In this study, *Escherichia coli*, *Proteus mirabilis*, and *Enterococcus* spp. strains were isolated at a rate of 68.8%.

On the basis of the comparisons that were made, we found that exposure of the bacteria to these chromogenic media does not alter their vital characteristics. The susceptibility tests carried out by taking samples of the colonies directly from the surface of the chromogenic media led to the same results as those obtained utilizing colonies taken directly from the standard medium (blood agar). A small number of minor discrepancies in zone inhibition size was observed; these variations in category occurred with antimicrobial agents that had zone-size

differences of only 1 mm. When the differences in zone-diameter sizes for organisms were compared by performing the standard method on 2 consecutive days to determine local intralaboratory variations, a similar range of errors was observed. The possibility of performing susceptibility testing directly from the chromogenic media, without any further loss of time due to subcultures in blood agar, makes the use of these media even more advantageous, as it enables a single medium to be used in the screening of clinical urine samples.

One additional factor to consider is the technical time and cost required for screening urine cultures with multiple types of media. We have estimated our costs at U.S. dollars (U.S.$) 0.55 for the conventional dual-plating method (CLED and TSA with 5% sheep blood or TSA with 5% sheep blood and MacConkey agar), U.S.$0.55 for CHR medium, and U.S.$0.44 for CPS medium. Furthermore, it seems clear that the application of a single medium (CHR or CPS medium) resulted in a 50% reduction in the time required for the processing of urine specimens and for the examination of cultures.

An important consideration in the assessment of these chromogenic media is the availability of technically skilled personnel to work with these media. We have found that, with a daily routine workload of about 100–150 urine samples, a technologist working on the bench can acquire the skills with these chromogenic media in 1 or, at most, 2 weeks. When this comparative evaluation of chromogenic media and conventional media was performed in our laboratory, due to the advantages of better performance and reduction in workload, our technologists clearly preferred to work with these chromogenic media.

Overall, CHR medium achieved a diagnostic yield that was slightly superior to that of the CPS medium. CHR medium produced a more intense colouration of the colonies and performed better in the presumptive identification of *Escherichia coli* and *Staphylococcus saprophyticus* strains and in differentiating the *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains, generally requiring a slightly lower number of biochemical tests for correct identification of the species (249 API/ID tests with CHR medium and 298 tests with CPS medium).

In conclusion, these chromogenic media can (i) reduce the number of media used for primary isolation to only one; (ii) enable a correct quantitative evaluation of the isolates; (iii) enable rapid presumptive identification of the most common bacteria responsible for urinary tract infections by means of colony colour, with only very few confirmatory tests required, which thereby reduces the need for subcultures and conventional identification tests; (iv) enable faster detection of mixed cultures; (v) prevent the swarming of *Proteus* and other motile bacteria; and (vi) enable susceptibility testing to be performed without the need for any further subcultures. The use of the chromogenic media described here can therefore significantly reduce the daily workload and decrease both the time expended and the related costs in favour of better performance and a more efficient use of available resources.

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Comparative Evaluation of Two Commercial Chromogenic Media for Detection and Presumptive Identification of Urinary Tract Pathogens

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Abstract The performance of two commercial chromogenic media for the isolation and presumptive identification of urinary tract pathogens, the CPS ID2 (bioMérieux, France) and the CHROMagar Orientation (BBL Becton Dickinson, USA), was evaluated and compared with that of cystine-lactose-electrolyte-deficient agar and tryptic soy agar with 5% sheep blood. The detection, determination of bacterial counts, and presumptive identification of bacteria causing urinary tract infections were evaluated in 3,000 urine specimens. The two chromogenic media showed excellent correlation with the standard media for the detection and the bacterial count of urinary pathogens. The *Escherichia coli* strains produced the expected colour on the CHROMagar Orientation and the CPS ID2 media in 99% and 90% of the cases, respectively. The *Klebsiella-Enterobacter-Citrobacter* and the *Proteus-Morganella-Providencia* groups were easily identified on both chromogenic media, but further biochemical tests were needed to differentiate them to a species level. Both media enabled the differentiation, with varying degrees of difficulty, of *Pseudomonas* spp. strains from members of the family *Enterobacteriaceae*. All isolates of *Enterococcus* spp. were correctly identified and were easily distinguished from the *Streptococcus agalactiae* isolates. *Staphylococcus saprophyticus* isolates were easy to identify only on the CHROMagar Orientation medium. No substantial difference was observed when comparing the results of the susceptibility tests, which were performed according to the standardized disk diffusion method as described by the National Committee for Clinical Laboratory Standards, for colonies recovered from the blood agar versus those recovered from the chromogenic media. In conclusion, the CPS ID2 and CHROMagar Orientation media enabled excellent detection, count determination, and presumptive identification of urinary pathogens, both in pure and mixed cultures, and reliable and accurate antimicrobial susceptibility testing directly from primary isolates. Moreover, these media allowed a remarkable reduction in the workload and a significant savings of time. On the basis of their performance, these media can replace the standard primary plating media used in the routine diagnosis of urinary tract infections.

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Introduction

The microbiology laboratory carries out a fundamental role in the diagnosis, therapy, and monitoring of urinary tract infections. A rapid identification of the etiological agent can provide the clinician with precious information regarding the appropriate choice of an antibiotic, even before the results of the susceptibility test are available [1]. Due to the high incidence of urinary tract infections, the number of relevant microbiological tests that are required account for a considerable workload in the daily routine of the laboratory. Culture is the testing method for screening urine samples, and the reference standard media include tryptic soy agar (TSA) with 5% sheep blood, MacConkey agar, and Columbia colistine-nalidixic acid agar [2, 3].

New chromogenic media have been developed in order to facilitate and speed up the presumptive identification of the main bacteria isolated from urinary samples [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. The presumptive identification is based on the different colourings of the bacterial colonies following the reactions between species-specific or genus-specific enzymes and the chromogenic substrates incorporated in the medium; only a few additional confirmatory tests are required.

Most patients with urinary tract infections need to be treated before the results of the susceptibility test are available. The choice of the appropriate drug depends on the identification and susceptibility of the microorganism. In order to perform the disk diffusion antibacterial susceptibility test as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), colonies isolated from a nonselective medium, such as blood

agar, must be utilized to prepare the suspension to be inoculated onto the Mueller-Hinton agar [16].

The objective of this study was to evaluate CPS ID2 (CPS) medium (bioMérieux, France) and BBL CHROMagar Orientation (CHR) medium (BBL Becton Dickinson, USA) for the detection, count determination, and presumptive identification of the bacteria responsible for urinary tract infections and compare them with two traditional control media: a cystine-lactose-electrolyte-deficient (CLED) medium and a tryptic soy agar (TSA) with 5% sheep blood. Additionally, the susceptibility tests performed on the colonies isolated from the two chromogenic media and the control medium (TSA with 5% sheep blood) were evaluated and compared. In order to standardize the readings of the zones of inhibition, we utilized a BioVideobact computerized instrument (Biokit, Spain) equipped with a video camera.

Materials and Methods

Urine Samples

Culture screening tests for the detection of bacteriuria were carried out on 3,000 random urine samples received consecutively at our microbiology laboratory. A total of 1,217 specimens were collected from outpatients, and 1,783 specimens were collected from patients hospitalized in different departments of our hospital as follows: 253 from the nephrology and the kidney transplant units, 202 from the haematology ward, 171 from the geriatric ward, 165 from the metabolic diseases department, 156 from the paediatric ward, 141 from the obstetrics and gynaecology ward, 139 from the urology ward, 123 from the internal medicine department, 102 from the surgery department, 97 from the intensive care unit, and 234 from other miscellaneous departments. Most of the urine specimens (2,710 from clean-catch midstream urine and 243 from indwelling bladder catheters) were collected in sterile plastic containers, while 57 urine specimens, the ones from paediatric patients, were collected with the application of a sterile plastic adhesive bag. All the samples were processed within 2 h after collection or were forwarded to the laboratory in refrigerated conditions, stored at 4°C, and processed within 4 h after collection.

Detection of Antimicrobial Agents

To detect the presence of antimicrobial agents in each urine sample, antimicrobial test plates were performed. A 6 mm diameter paper disk was dipped into the urine sample and placed onto a Mueller-Hinton agar plate seeded with a fully sensitive strain of *Staphylococcus epidermidis* (ATCC 12228). Plates were incubated at 35–37°C in aerobic conditions and examined after 18–24 h for zones of inhibition around the disk. Antimicrobial substances were detected in 405 urine specimens. Of these specimens, 203 yielded clinically significant bacteriuria and 202 yielded nonsignificant bacteriuria or no growth.

Microscopy

For each of the urine samples, a microscopic examination and cell count were performed on well-mixed uncentrifuged urine samples by using Kova slides with 10 counting grids. Each of the 10 chambers holds a standardized 6.6 µl volume of the sample. A sample was considered positive for the presence of pyuria when the count was ≥10 leucocytes/µl. Squamous epithelial cells were recorded as "few" (<5 cells per low-power field), "moderate" (6–15 cells per low-power field), or "numerous" (>15 cells per low-power field). The presence of bacteria was also recorded. Microorganisms were classified for morphology, and the approximate number per high-power field was quantified.

Test Media Used and Inoculation Technique

The CPS medium (bioMérieux, France) and the CHR medium (Becton Dickinson, USA) are sold as prepared plated media. The TSA medium (Difco Laboratories, USA) and the CLED medium (Becton Dickinson, USA) were prepared from dehydrated powder, according to the instructions provided by the manufacturer. For the preparation of blood plates, 5% sterile defibrinated sheep blood was added to sterile agar that previously had been melted and cooled to 45–50°C. The media were dispensed in 90 mm diameter sterile petri capsules, which were stored at 4°C and utilized within 4 weeks. Urine samples were directly inoculated in parallel conditions on four media with a 0.0075 ml calibrated loop by means of a semiautomatic Urine Robot (p.b.i. International, Italy). For determination of the bacterial count, the volume of the "nonstandard" calibrated loop supplied with the Urine Robot was converted to 0.01 ml using a conversion factor (1 colony equals 133 colony-forming units [cfu]/ml). This system was used in order to guarantee standardization of the inoculating process; the technique employed was the standard surface-streak method. The plates were then incubated at 37°C for 18–20 h [2, 3].

Quality Control

Each batch of medium prepared in the laboratory, or a new lot of the two commercial media, was tested for sterility, ability to support the growth, and chromogenic and biochemical response with American Type Culture Collection (ATCC) control strains. The *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 strains were inoculated in brain heart infusion broth (BBL Becton Dickinson) and incubated for 4–5 h. After adjusting the turbidity level to the standard of 0.5 McFarland, the suspensions, previously diluted 1:100 in physiological solution, were inoculated on the surface of the media by means of the Urine Robot calibrated loop.

Interpretation of Results

Guidelines for processing urine samples in our laboratory have been established based on Cumitech 2B [2] and on a close cooperation between the clinical and laboratory staff. The following criteria were adopted for the microbiological assessment of urine specimens: (i) a colony count of $\geq 10^5$ cfu/ml of one or two species of probable pathogens, with the possible presence of other isolates in low concentrations ($\leq 10^4$) that were ignored, as the criterion for diagnosis of pyelonephritis, acute cystitis, and asymptomatic bacteriuria; (ii) a colony count of 10^2–10^5 cfu/ml of one or two species of probable pathogens from symptomatic patients (women with frequency-dysuria syndrome, symptomatic males, and selected urology, paediatric, and geriatric patients), from patients with indwelling bladder catheters, from urine specimens collected by in-and-out (straight) catheterization or suprapubic aspiration and from other specimens for which special cultures are recommended [2].

Patients with complicated infections (associated with stones, renal abscesses, or long-indwelling urinary catheters) often will have multiple organisms in their urine. As many as four to five or more different species may be recovered from patients on long-term catheter drainage, and the clinical microbiology laboratory should be alerted to the possibility of polymicrobic bacteriuria to avoid rejecting the specimen as contaminated. Nevertheless, when possible, it is preferable to collect urine specimens after placement of a new catheter rather than from a catheter that has been in place for an extended time, because the latter often develops a microbial flora different from that present within the patient's urinary tract, in which case urine specimens from the collecting tubing may not reflect the microbiological status of the patient's urinary tract [2].

Detection of a bacterial count of $<10^4$ cfu/ml in nonselected patients devoid of clinical information was interpreted as nonsignificant bacteriuria; detection of sterile cultures was evaluated as no growth. Both of these types of cultures were considered negative. Detection of more than two species of bacteria in low concentrations from urine specimens was interpreted as a contaminated (mixed) culture.

The counts obtained from the analysis of the two chromogenic media were compared with those obtained with the CLED medium. CLED is a differential, nonselective medium that favours the growth of most of the pathogenic urinary bacteria. It provides sufficient differentiation between the colonies and enables the detection of lactose-fermenting gram-negative enteric bacteria. It inhibits the swarming of *Proteus* and other motile bacteria. TSA with 5% sheep blood was used predominantly for the detection of urinary pathogens, in particular the more fastidious bacteria, but not for determining the count because it has several disadvantages, such as the minimal differentiation between the organisms recovered and its complete failure to inhibit the swarming of *Proteus* and other motile bacteria.

Presumptive Identification of Bacteria

CPS and CHR are culture media containing specific chromogenic substrates for particular enzymes such as β-glucuronidase on CPS medium and β-galactosidase on CHR medium, which indicate the development of colonies that are coloured from pink to bordeaux, and β-glucosidase enzyme, which indicates the development of colonies coloured from blue to greenish-blue on both media. With these two media, it is also possible to utilize two supplementary reagents in order to detect two other enzyme-related activities: tryptophanase (indole test) and tryptophan deaminase. The activity of the tryptophan deaminase enzyme is characteristic for the *Proteus-Morganella-Providencia* group and, when this activity ranges from medium to strong, there is a spontaneous and clearly visible production of a diffuse brown pigment that colours the medium around the colonies on both media, even without utilizing a specific reagent ($FeCl_3$). According to the manufacturer's instructions, the media enable the identification of the most common microorganisms involved in urinary tract infections as follows: (i) *Escherichia coli* isolates produce a pink to bordeaux colony colour; a confirmatory test for a definitive identification must be performed by detection of indole production (indole test); (ii) *Enterococcus* spp. isolates produce small colonies with blue to turquoise colour, and a microscopic examination is required to confirm the morphology of the organisms (cocci in chains). On CPS medium, the colony colour and the microscopic evaluation allow the identification of *Enterococcus* spp., while on CHR medium, identification is confirmed by an L-pyrrolidonyl-β-naphthalamide hydrolysis test; (iii) *Proteus mirabilis* isolates produce colourless or beige colony colour, surrounded by a brown halo, and a confirmatory test for a definitive identification must be performed by detection of indole production using a drop of Kovac's reagent. *Proteus penneri* isolates are indistinguishable from *Proteus mirabilis* isolates (both indole negative) on chromogenic media. Nevertheless, *Proteus penneri* strains are occasionally isolated from urine specimens, and they are chloramphenicol resistant and can be easily distinguished from *Proteus mirabilis* strains by an antimicrobial susceptibility test.

The two media also enable the presumptive identification of the following: (i) the *Klebsiella-Enterobacter-Serratia* group, which develop colonies that are coloured from metallic blue to blue-green; a microscopic evaluation is required to confirm morphology (bacilli); and (ii) the *Morganella-Proteus* indole-positive-*Providencia* group, whose colonies are colourless, beige, or blue-green, surrounded by a brown halo, and produce a positive indole reaction. The latter two groups of bacteria, however, require further biochemical tests for correct identification of the species.

CHR medium is also claimed to allow the presumptive identification of *Staphylococcus saprophyticus*, on the basis of the light-pink colour of the colonies and a microscopic examination, which is required to confirm the morphology (cocci).

The indole test on CPS (ID indole; bioMérieux) and on CHR (DMACA Indole Dropper reagent; BBL Becton Dickinson) can be carried out on filter paper. Further confirmatory tests, such as catalase (3% H_2O_2), the Slide Staph-Kit (bioMérieux), the Streptex acid extraction kit (Murex Diagnostics, France), and the oxidase (N, N, N′, N′-tetramethyl-p-phenylendiamine dihydrochloride; Carlo Erba, Italy) tests were carried out according to the manufacturer's instructions. In all cases of dubious results, or whenever further confirmatory identification was required, system identification profiles were used as follows: ID 32 E for *Enterobacteriaceae*; API 20 NE for nonfermenting gram-negative rods; ID 32 Staph for staphylococci; API 20 Strep for streptococci; and ID 32 C for the yeasts (bioMérieux).

Antimicrobial Susceptibility Testing

A performance standard for antimicrobial disk susceptibility testing was carried out on 200 gram-negative isolates and 100 gram-positive isolates, taking samples of the colonies directly from the blood agar according to the NCCLS guidelines [16]. Antimicrobial susceptibility tests of the same bacteria were prepared concurrently by taking colonies from the CHR medium and from the CPS medium. The results of the three tests were evaluated by means of computerized readings of the three corresponding plates, using the instrument BioVideobact (Biokit), which guarantees standardized readings of the zones of inhibition that are produced on the surface of the Mueller-Hinton medium. The readings were evaluated in accordance with the guidelines indicated in the NCCLS documents M2-A6 [16] and M7-A4 [17]. BioVideobact automatically calculates the size of the zones of inhibition by means of a video camera, determines the category of interpretation, and through the interpolation with a specific regression line, it also calculates the minimum inhibitory concentration.

Results

In this study, a routine examination of 3,000 urine specimens was carried out for microbiological evaluation; 1,694 cultures were devoid of bacterial growth, while 381 cultures developed clinically nonrelevant bacteria ($<10^4$ cfu/ml) or developed more than two species in low concentrations. The remaining 925 (30.8%) urine specimens were considered positive and clinically significant. A single bacterial species was isolated in 829 of these samples, and two bacterial species were isolated in 96 specimens. In all cultures of the latter group, both isolates developed a significant colony count. A total of 1,021 bacterial strains were isolated, 954 with a bacterial count $\geq 10^5$ cfu/ml and 67 with a bacterial count between $\geq 10^2$ and $<10^5$ cfu/ml (Table 1).

The fertility of the media was evaluated on the basis of the plate readings, which were carried out after 18–20 h of incubation. Complete correspondence in the results on both chromogenic media was detected, proving that both media were able to support the growth of all the isolates. Valid results were also obtained with regard to the bacterial count, except for five cultures (3 that contained strains of *Candida* spp., 1 that contained *Enterococcus* spp. growing on CPS medium, and 1 contained *Enterococcus* spp. strain growing on CHR medium), all of which contained bacterial counts of $<10^5$ cfu/ml, while on the corresponding chromogenic medium and on the CLED medium, the bacterial count was $\geq 10^5$ cfu/ml.

Table 1 Number of clinically important isolates recovered from 925 urine samples

Species	Total strains isolated	No. of clinically important strains isolated					
		CHR medium		CPS medium		CLED medium	
		≥10^5 cfu/ml	<10^5 cfu/ml	≥10^5 cfu/ml	<10^5 cfu/ml	≥10^5 cfu/ml	<10^5 cfu/ml
Acinetobacter spp.	2	2	0	2	0	2	0
Candida spp.	31	27	4	24	7	27	4
Citrobacter spp.	16	16	0	16	0	16	0
Escherichia coli[a]	429	392	32	397	32	386	32
Enterobacter spp.	17	17	0	17	0	17	0
Enterococcus spp.	213	201	12	201	12	202	11
Hafnia alvei	3	3	0	3	0	3	0
Klebsiella spp.	96	87	9	87	9	87	9
PMP group[b]	89	87	2	87	2	87	2
Pseudomonas spp.	57	48	9	48	9	48	9
Salmonella spp.	1	1	0	1	0	1	0
Streptococcus agalactiae	36	36	0	36	0	36	0
Serratia marcescens	6	6	0	6	0	6	0
Staphylococcus spp.	25	25	0	25	0	25	0
Total	1,021	948	68	950	71	943	67

[a] Eleven urine samples on CPS medium and five urine samples on CHR medium with a double bacterial isolation contained two *Escherichia coli* variants, but only one strain was identified on CLED medium

[b] *Proteus-Morganella-Providencia* group

Table 2 lists the different bacterial species that were isolated and the colour and morphological characteristics of the corresponding colonies on the two chromogenic media. *Escherichia coli* was the predominant species (429 isolates). On the CHR medium, 425 strains developed colonies with the characteristic pink colour; only four strains failed to produce a chromogenic reaction, showing beige colonies. On the CPS medium, 386 strains developed colonies with the expected pink-to-bordeaux colour, while 43 strains were negative β-glucuronidase, producing colonies from colourless to beige. Of all 429 *Escherichia coli* isolates, 3 exhibited a negative indole reaction, 2 of which produced beige colonies on both chromogenic media.

Eleven cases of double bacterial isolation were found in which the two isolates were identified as two different strains of *Escherichia coli*. These strains were not detected on CLED or TSA with 5% sheep blood, but they were clearly identifiable due to the different colouring characteristics of the colonies on CPS medium (pink to bordeaux or pale pink, colourless, beige) in all 11 cases and on CHR medium (pink or pale pink, beige) in 5 cases. These two variants of *Escherichia coli* showed some differences in susceptibility patterns that resulted in a significant change in both the interpretation of the susceptibility test results (S↔R) and in the standard biochemical profiles used to identify the strains correctly. These differences confirmed the presence of two different *Escherichia coli* strains responsible for urinary tract infections.

All *Enterococcus* spp. isolates (n=213) were correctly detected based on the appearance of typical small blue-to-turquoise colonies (β-glucosidase positive) and by microscopic examination (cocci in chains) on both chromogenic media; no false-negative results were obtained. An additional L-pyrrolidonyl-β-naphthalamide hydrolysis test, performed to confirm precise genus identification on CHR medium, was positive for all 213 strains that yielded small blue-to-turquoise colonies and cocci in chains; no false-positive results were obtained. These media allow easy differentiation of a mixed culture of *Enterococcus* spp. and members of the *Klebsiella-Enterobacter-Serratia* group because of the different colour and size of the colonies. Furthermore, *Enterococcus* spp. isolates are nearly always easy to differentiate from *Streptococcus agalactiae* isolates (n=36). The *Streptococcus agalactiae* isolates produced light-blue pin-like colonies on CHR and white, light-blue-to-turquoise pin-like colonies on CPS medium, except for four strains that produced pink-coloured colonies on the latter medium.

Both chromogenic media performed as well as the standard reference media in supporting the growth of *Staphylococcus* spp. isolates. In this study, the following species were isolated: *Staphylococcus aureus* (n=10), *Staphylococcus epidermidis* (n=4), *Staphylococcus saprophyticus* (n=6), *Staphylococcus simulans* (n=2), *Staphylococcus warneri* (n=2), and *Staphylococcus haemolyticus* (n=1). *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates produced colonies that ranged from yellow-golden to opaque-white colour. *Staphylococcus saprophyticus* isolates produced pink-coloured colonies on CHR medium and white-coloured colonies on CPS medium. *Staphylococcus simulans* isolates produced pink-coloured colonies on CHR medium, which could not be distinguished from colonies of the *Staphylococcus saprophyticus* isolates, while *Staphylococcus warneri* isolates and *Staphylococcus haemolyticus* isolates produced pink-coloured colonies on CPS medium.

The white, creamy, convex colonies of *Candida albicans* were difficult to differentiate from the *Candida* spp. isolates on CHR medium, while on the CPS medi-

Table 2 Colour of colonies on CHR medium and on CPS medium

Organism	Total no. of isolates	CHR medium		CPS medium	
		No. (%) of isolates with described colour	Description of pigment and/or morphology of colonies	No. (%) of isolates with described colour	Description of pigment and/or morphology of colonies
Acinetobacter spp.	2	2 (100)	beige	2 (100)	beige
Candida spp.	31	31 (100)	white, creamy, convex	31 (100)	white, creamy, convex
Citrobacter spp.	16	14 (87.5)	metallic blue with or without pink halo	10 (62.5)	blue-green
		2 (22.5)	pink	6 (37.5)	beige
Escherichia coli	429	425 (99)	pink	386 (90)	pink to bordeaux
		4 (1)	beige	43 (10)	colourless to beige
Enterobacter spp.	17	17 (100)	metallic blue with or without pink halo	17 (100)	blue-green
Enterococcus spp.	213	213 (100)	blue to turquoise, small	213 (100)	blue to turquoise, small
Hafnia alvei	3	2 (66.7)	beige	3 (100)	beige
		1 (33.3)	pink with blue halo		
Klebsiella spp.	96	96 (100)	metallic blue with or without pink halo	96 (100)	blue-green
Morganella morganii	7	7 (100)	colourless to beige with brown halo	7 (100)	colourless to beige with brown halo
Proteus mirabilis	61	61 (100)	beige with brown halo	61 (100)	beige with brown halo
Proteus vulgaris	5	3 (60)	beige with brown halo	5 (100)	beige with brown halo
		2 (40)	blue-green with brown halo		
Providencia spp.	16	16 (100)	beige with brown halo	16 (100)	beige with brown halo
Pseudomonas spp.	57	53 (93)	transparent, yellow to green serrated edge, diffused	35 (61.5)	transparent, yellow to green serrated edge, diffused
		4 (7)	beige with or without green halo	22 (38.5)	beige with or without green halo
Salmonella spp.	1	1 (100)	beige	1 (100)	beige
Streptococcus agalactiae	36	36 (100)	light blue, pin-like	32 (88.9)	white, light blue, pin-like
				4 (11.1)	pink
Serratia marcescens	6	6 (100)	blue-green	3 (50)	blue-green
				3 (50)	beige
Staphylococcus saprophyticus	6	6 (100)	pink opaque	6 (100)	white
Staphylococcus spp.	19	19 (100)	golden opaque, white, pink	19 (100)	golden opaque, white, pink

um, the isolates of the latter group showed poor growth and developed very small colonies that were not always easy to see.

Six *Lactobacillus* spp. isolates produced blue-coloured colonies on CHR medium, with a count $\geq 10^5$ cfu/ml; these colonies did not develop on CPS medium.

The zone diameters in the standardized disk diffusion test, from blood agar and from the two chromogenic media, were measured and the distributions of the differences in the zones of inhibition of each antimicrobial agent were recorded. From the comparison of the results obtained in the susceptibility test carried out by taking samples of the colonies from the three different media, a nearly complete concordance was noted. Two minor errors (I↔R) were noted when comparing the tests carried out on gram-negative bacteria, and two minor errors (I↔R) were likewise noted when comparing the tests carried out on gram-positive bacteria.

Discussion

The conventional method for the microbiological diagnosis of urinary tract infections is the quantitative culture of urine samples on standard solid media. The need to introduce new technological solutions in this method has become indispensable for two reasons. First, only a minor percentage (20–30%) of the large number of urine samples that the microbiology laboratory must analyze every day are infected; thus, a remarkable amount of time and culture media is wasted on the evaluation of samples that are mostly clinically nonsignificant. Secondly, there is a need for rapid preliminary identification of the etiological agent in order to enable the clinician to begin the appropriate therapy before the results of susceptibility testing are available. Both the laboratory results and the therapeutic regimen must therefore be available in a shorter period of time. Undoubtedly, an

important aspect of every study is the proven validity of the method, which is essential in order to maintain high-quality, reliable diagnostics.

In an attempt to meet the above requirements, the performances of commercially available CHR and CPS chromogenic media were evaluated and compared with one another as well as with other standard media employed in the routine work of a clinical microbiology laboratory. Our data showed the excellent ability of both chromogenic media to detect the different species of urinary tract pathogens, to support the growth of all the isolates, and to evaluate their growth quantitatively; up to now, the combined use of two conventional media has been required to achieve detection, growth, and quantitative assessment. Unlike blood agar, these chromogenic media inhibit or significantly decrease the swarming of *Proteus*, *Escherichia coli*, and *Klebsiella* mucosal strains, thus enabling a bacterial count and a correct identification of the colonies to be obtained.

The most common microorganisms that produce urinary tract infections are gram-negative bacteria, which belong to the *Enterobacteriaceae* family. CHR and CPS media used different chromogenic substrates to detect specific bacterial enzymes, which allow identification of *Escherichia coli*. Presumptive identification of *Escherichia coli* strains, which accounted for 42% of the clinically significant isolates in our study, was better on CHR medium (99% identified), which detects β-galactosidase enzyme activity, than on CPS medium (90% identified), which detects β-glucuronidase enzyme activity. This difference is statistically significant ($P<0.001$, chi-square test). The superior sensitivity of β-galactosidase enzyme activity in comparison with β-glucuronidase enzyme activity for identification of *Escherichia coli* has recently been reported [6, 14].

In contrast, a better detection of multiple *Escherichia coli* variants in the same urine specimen was observed on CPS medium when compared to CHR medium. This finding is in accordance with the results of the study of Carricajo et al. [14]. Only the chromogenic media enabled detection of these *Escherichia coli* variants, and a correct differentiation is particularly important in order to establish suitable antibiotic treatment.

On these chromogenic media, a correct identification of all pink-to-bordeaux colonies must be confirmed by an indole test, since some strains of *Salmonella* spp., *Citrobacter freundii*, and *Shigella sonnei* also produce β-glucuronidase or β-galactosidase activity, showing pink-to-bordeaux colonies [6, 8, 9, 10]. However, most of these strains are isolated only occasionally from urine specimens and are indole negative. Nevertheless, some strains of *Citrobacter diversus* may also yield pink-to-red colonies on CHR medium and produce a positive indole test result, leading to misidentification as *Escherichia coli* strains (Table 2).

Enterococci were correctly identified on both chromogenic media at the genus level. The two most common enterococcal species, *Enterococcus faecalis* and *Enterococcus faecium*, showed similar colony colour and were indistinguishable at the species level, which is in accordance with the results of other reports [6, 9]. In our study, enterococci represented the second most common cause of urinary tract infection (20.9%); they also caused nosocomial infections and most often were present in catheter-associated cystitis.

All of the *Proteus mirabilis* isolates were chromogenically distinct on both chromogenic media, which yielded the same rate of detection for all isolates of the *Proteus-Morganella-Providencia* group. However, five of the indole-negative isolates of *Proteus mirabilis* were misidentified as indole producers when these strains were isolated in association with an indole-positive strain of *Escherichia coli* or *Klebsiella pneumoniae* var. *oxytoca*. The possibility that the metabolite of the tryptophanase can spread in the medium was suggested previously [8, 14], and for correct identification, it is recommended that all strains detected in mixed cultures that contain at least one indole-positive isolate be confirmed by conventional biochemical tests.

The *Klebsiella*, *Enterobacter*, and *Citrobacter* isolates were easily identified by the production of large metallic-blue and blue-green colonies on CHR and CPS chromogenic media, but further biochemical tests were needed to differentiate them to a species level.

The *Pseudomonas* spp. strains were differentiated and chromogenically distinguished, with varying degrees of difficulty, from the *Enterobacteriaceae* members on both chromogenic media, but further biochemical tests were needed for a correct identification at the species level. Only the CHR medium enabled correct identification of the *Staphylococcus saprophyticus* isolates, which produced typical pink-coloured colonies, as confirmed by previous reports [6, 11]. In our study, two *Staphylococcus simulans* isolates also produced pink-coloured colonies.

The routine processing of urine samples requires that the laboratory staff be able to evaluate plates with mixed cultures, because often the absence of particular differentiating characteristics will hinder the different bacterial strains from being recognized. The differences in colour and size of the colonies on these two chromogenic media enabled easy differentiation of the isolates in mixed cultures.

The well-distinguished colours produced by some of the species enable rapid presumptive identification of the main bacteria responsible for urinary tract infections; only a few additional confirmatory tests are required. In this study, *Escherichia coli*, *Proteus mirabilis*, and *Enterococcus* spp. strains were isolated at a rate of 68.8%.

On the basis of the comparisons that were made, we found that exposure of the bacteria to these chromogenic media does not alter their vital characteristics. The susceptibility tests carried out by taking samples of the colonies directly from the surface of the chromogenic media led to the same results as those obtained utilizing colonies taken directly from the standard medium (blood agar). A small number of minor discrepancies in zone inhibition size was observed; these variations in category occurred with antimicrobial agents that had zone-size

differences of only 1 mm. When the differences in zone-diameter sizes for organisms were compared by performing the standard method on 2 consecutive days to determine local intralaboratory variations, a similar range of errors was observed. The possibility of performing susceptibility testing directly from the chromogenic media, without any further loss of time due to subcultures in blood agar, makes the use of these media even more advantageous, as it enables a single medium to be used in the screening of clinical urine samples.

One additional factor to consider is the technical time and cost required for screening urine cultures with multiple types of media. We have estimated our costs at U.S. dollars (U.S.$) 0.55 for the conventional dual-plating method (CLED and TSA with 5% sheep blood or TSA with 5% sheep blood and MacConkey agar), U.S.$0.55 for CHR medium, and U.S.$0.44 for CPS medium. Furthermore, it seems clear that the application of a single medium (CHR or CPS medium) resulted in a 50% reduction in the time required for the processing of urine specimens and for the examination of cultures.

An important consideration in the assessment of these chromogenic media is the availability of technically skilled personnel to work with these media. We have found that, with a daily routine workload of about 100–150 urine samples, a technologist working on the bench can acquire the skills with these chromogenic media in 1 or, at most, 2 weeks. When this comparative evaluation of chromogenic media and conventional media was performed in our laboratory, due to the advantages of better performance and reduction in workload, our technologists clearly preferred to work with these chromogenic media.

Overall, CHR medium achieved a diagnostic yield that was slightly superior to that of the CPS medium. CHR medium produced a more intense colouration of the colonies and performed better in the presumptive identification of *Escherichia coli* and *Staphylococcus saprophyticus* strains and in differentiating the *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains, generally requiring a slightly lower number of biochemical tests for correct identification of the species (249 API/ID tests with CHR medium and 298 tests with CPS medium).

In conclusion, these chromogenic media can (i) reduce the number of media used for primary isolation to only one; (ii) enable a correct quantitative evaluation of the isolates; (iii) enable rapid presumptive identification of the most common bacteria responsible for urinary tract infections by means of colony colour, with only very few confirmatory tests required, which thereby reduces the need for subcultures and conventional identification tests; (iv) enable faster detection of mixed cultures; (v) prevent the swarming of *Proteus* and other motile bacteria; and (vi) enable susceptibility testing to be performed without the need for any further subcultures. The use of the chromogenic media described here can therefore significantly reduce the daily workload and decrease both the time expended and the related costs in favour of better performance and a more efficient use of available resources.

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