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 Oxytetracycline (BD, Becton Dickinson, USA), was evaluated and compared with that of ceftriaxone-lectin-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 Oxytetracycline and the CPS ID2 media in 99% and 99% of the cases, respectively. The Klebsiella-Enterobacter-Carbapath and the Proteus-Morganella-Providence 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 Enterococcus 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 Oxytetracycline medium. No substantial difference was observed when comparing the results of the susceptibility tests, which were performed according to the standard 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 Oxytetracycline 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 saving 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.

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 colistin-salmonella-sodium-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 urine 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.
agars, must be utilized to prepare the suspension; to be in-
oculated on the Muller-Hinton agar [14].

The objective of this study was to evaluate CPS D2 (CPS) medium (BioMérieux, France) and BBL CHROM- 
agar Oxidase (CHROM) medium (BBL, Becton Dickin-
son, USA) for the detection, count determination, and
presumptive identification of the bacteria responsible for
urinary tract infections and compare them with two tradi-
tional control media: a cystine-lactose- electrolyte-defi-
cient (CLED) medium and a tryptic soy agar (TSA) with
5% sheep blood. Additionally, the susceptibility tests
performed on the colonies isolated from the two chromo-
genic 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 BioVideocontact computerized instrument (Bio-
kit, Spain) equipped with a video camera.

Materials and Methods

Urine Samples

Culture screening was done for the detection of bacteria wort collected out on 3,000 random, urine samples received consecutively at our microbiology laboratory. A total of 1,217 samples were collected from outpatients, and 1,783 specimens were collected from pa-
tients hospitalized in different departments of our hospital as fol-
lows: 253 from the nephrology and the kidney transplant units, 202 from the hematology ward, 171 from the gynecologic ward, 165 from the otorhinolaryngologic department 156 from the pediatric ward, 141 from the obstetrics and gynecology ward, 119 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. More of the urine specimens (7,716 from clean-catch midstream urine and 243 from indwelling bladder catheters) were collected in sterile plastic bags,
while 57 urine specimens, the ones from pediatric pa-
tients, were collected with the application of a sterile plastic adhe-
sive bag. All the samples were processed within 2 h after collec-
tion 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 specimen, antimicrobial test plates were performed. A 6 mm diameter paper disk was placed onto a MacConkey
agar plate seeded with a fully sensitive strain of Staphylococcus
aureus (ATCC 29212). Paper disks were incubated at 35-37°C in
anaerobic conditions and examined after 18-24 h for zones of inhibition
around the disk. Antimicrobial substances were defined as 405 urine
specimens. Of these specimens, 201 yielded clinically significant bac-
terium and 202 yielded nonsignificant bacteria or no growth.

Microscopy

For each of the urine samples, a microscopic examination and cell
counts were performed on well-mixed supernatant urine samples
by using Kova slides with 10 counting grids. Each of the 10 cham-
bors holds a standardized 6.6 ml volume of the sample. A sample
was considered positive for the presence of trypsin when the count
was 210 micromorphoses. Squamous epithelial cells were counted as
"true" (5 cells per low-power field), "moderate" (5-15 cells per
low-power field), or "mucinous" (>15 cells per low-power field)
. The presence of bacillus was also recorded. Microcoagulates were
classified for morphology, and the approximate number per high-
power field was estimated.

Test Media Used and Inoculation Technique

The CPS medium (BioMérieux, France) and the CHROM medium (Bec-
ton Dickinson, USA) are used as streaked plated media. The TSA medium (Difco Laboratories, USA) and the CHROM medium (Becton Dickin-
don, USA) were prepared from defibrinated powder, according to
the instructions provided by the manufacturer. For the preparation of
Blood plasma, 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 wells capsules,
which were stuffed at 4°C and utilized within 4 weeks. Urine samples
were directly processed in parallel conditions on these media with a
0.007 ml inoculated loop by means of a semiautomated Urine Robot
(Becton Dickinson, Italy). For determination of the bacterial count,
the volume of the "nonstructured" cellulose loop applied with the
Urine Robot was converted to 0.1 ml using a conversion factor (1
colony equates 133 colony-forming unit [cfu]/ml). This system was
used in order to guarantee standardization of the inoculating process.

This technique employed was the standard membrane method. The
plates were then incubated at 37°C for 18-24 h.[2]

Quality Control

Each batch of medium prepared in the laboratory, or a new lot of
the commercial media, was tested for sterility, ability to sup-
port the growth and chromogenic and biochemical response with
American Type Culture Collection (ATCC) stored strains. The
Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC
13883, Staphylococcus aureus ATCC 29453, Proteus mirabilis STCU
29212, Pseudomonas aeruginosa ATCC 27853, and
Candida albicans ATCC 10231 were kept in brain heart infusion broth (BBL, Becton Dickinson) and incubated for
5-6 h. After adjusting the turbidity level to the standard of 0.5
McFarland, the suspensions, previously diluted 1:100 in physi-
ological 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 Cerberus 2B [2] and on a close cooper-
ation between the clinical and laboratory staff. The following crite-
ria were adapted for the microbiological assessment of urine speci-
mens: (a) a colony count of 2x10^6 cfu/ml of one or two species of
probable pathogens, with the possible presence of other isolates in
low quantities (<1000) that were ignored, as the criteria for di-
agnosis of pyelonephritis, acute cystitis, and asymptomatic bacteri-
uria; (b) a colony count of 10^-10^-1 cfu/ml of one or two species of
probable pathogens from symptomatic patients (women with fre-
quency, urgency, strong discomfort, strangury, and lower uri-
ology, pediatric, and genital patients), from patients with indwelling
bladder catheters, from urine specimens collected by in-dwelling
(strength catecholamine or sympathic agonist and from other
specimens for which special culture 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 five to nine
more different species may be isolated from patients on long-
term catheter drainage, and the clinical microbiology laboratory
should be alerted to the possibility of polymicrobial bacteriuria in
avoid repeating the specimen as uncontaminated. Nevertheless, when
possible, it is preferable to collect urine specimens after placement of
a new catheter to rule out a catheter infection 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 specimen from the collecting tubing may not reflect the
microbiological status of the patient's urinary tract.[2]
Detection of a bacterial count of <10<sup>6</sup> cfu/ml in nonselected patient devoid of clinical information was interpreted as nonsignificant haemorrhagic 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 con- taminates from sterile specimens was interpreted as a contaminat- ed (ms)<sup>2</sup> culture.

The counts obtained from the analysis of the two chromogenic media were compared with those obtained with the CLED medi- um. CLED is a differential, nonselective medium that favors the growth of most of the pathogenic cutaneous bacteria. It provides suffi- cient differentiation between the colonies and enables the distinc- tion of lactose-refering (Lactobacillus) and non-lactose-refering bacteria. It inhib- its the swarming of Proteus and other motile bacteria. TSI weak.

Pressurized Identification of Bacteria
CPS and OIR are culture media containing specific chromogenic substrates for particular enzymes such as B-glucuronidase on CPS medium and β-galactosidase in OIR medium, which indicate 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 re- agents in order to detect two other enzyme-related activities: try- pohemolysin (insole test) and tyrosine deaminase. The activity of the tryptophan deaminase enzyme is characteristic for the Proteus- Morganella-Proteellia group and, when this activity ranges from medium to strong, there is a spontaneous and clearly visible pro- duction of a yellowish brown pigment that colours the medium around the colonies. The test for tryptophan deaminase is a qualitative test for a specific en- zyme (PDC), according to the manufacturer's instructions, the medium is inoculated with the test culture and after 24h of incubation a specific microorgan- isms involved in staphylococcal infections as follows: (i) Escherichia coli, a gram-negative bacillus that causes urinary tract infections; a confirmatory test for a definitive identification must be performed by detects- ting of indole production (insole test); (ii) Enterococcus spp. isolates produce small colonies with blue to indigo colour; and a macroscopic examination is required to confirm the morphology of the organism (insole colour). On CPS medium, the colony col- our and the macroscopic evolution allow the identification of En- terococcus spp., where on CLED medium the identification is estab- lished by E. Lysinophorilum and E. spinitennis isolates hydrolysed test. (iii) Proteus vulgaris was used predominantly for small colonies or large colony cut- out, surrounded by a brown halo, and a confirmatory test for a de- finitive identification must be performed by detecting of indole production using a drop of Kovac's reagent. Proteus pueater iso- lates were detected by using a drop of Kovac's reagent on Proteus vulgaris isolates (both, insole negative) on chromogenic media. No-organisms, Proteus pue- arter strains are occasionally isolated from x ray specimens, and they are chromophenol resistant and can be easily distinguished from Proteus species thanks to an antimicrobial susceptibility test.

The two media also enable the presumptive identification of the following: (i) the Klebsiella-Enterobacter-Serratia group, which develops colonies that tests coloured from metallic blue to blue-green; a macroscopic evolution is related to the green morphology (ms); and (ii) the Morganella-Proteus isolate-positive group, whose colonies are colourless, or blue- green, surrounded by a brown halo, and produce a poxii or indigo reaction. The latter two groups of bacteria, however, require fur- ther biochemical tests for correct identification of the species.

CLED medium is also claimed to allow the presumptive identifi- cation of Staphylococcus aureus and Staphylococcus epidermidis, on the basis of the light pink colony of the colonies and a macroscopic excretion, which is required to confirm the morphology (ms).
Table 1 Number of clinically important isolates recovered from 923 urine samples

<table>
<thead>
<tr>
<th>Species</th>
<th>Total strains isolated</th>
<th>No. of clinically important strains isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHR medium</td>
<td>CPS medium</td>
</tr>
<tr>
<td></td>
<td>≥10^6 cfu/ml</td>
<td>&lt;10^6 cfu/ml</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Camidia spp.</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>Chlostridium spp.</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>429</td>
<td>392</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>E. coli</td>
<td>273</td>
<td>261</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>PMG group^p</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>1,021</td>
<td>948</td>
</tr>
</tbody>
</table>

^p Previus-Morganella-Prevotella 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, 7 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 the 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=4), 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-glucuronidase positive) and by microscopic examination (cocc in chains) on both chromogenic media. No false-negative results were obtained. An additional L-tryptophan-β-naphthalamide hydrolysis test, performed to confirm precise genus identification on CHR medium, was positive for all 215 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* groups 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 to pin-like colonies on CHR medium, while light-blue-to-turquoise to 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 simulans* isolates, while *Staphylococcus warneri* isolates and *Staphylococcus haemolyticus* isolates produced pink-coloured colonies on CPS medium.

The white, creamy, convex colonies of *Camidia albi- can* were difficult to differentiate from the *Camidia* spp. isolates on CHR medium, while on the CPS medi-
<table>
<thead>
<tr>
<th>Organism</th>
<th>Total no. of isolates</th>
<th>CHR medium</th>
<th>Description of pigment and/or morphology of colonies</th>
<th>CPS medium</th>
<th>Description of pigment and/or morphology of colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aerococcus spp.</em></td>
<td>2</td>
<td>2 (100)</td>
<td>beige</td>
<td>2 (100)</td>
<td>beige</td>
</tr>
<tr>
<td><em>Candida spp.</em></td>
<td>31</td>
<td>31 (100)</td>
<td>white, creamy, convex</td>
<td>31 (100)</td>
<td>white, creamy, convex</td>
</tr>
<tr>
<td><em>Clostridium spp.</em></td>
<td>16</td>
<td>14 (87.5)</td>
<td>magenta blue with or without pink halo</td>
<td>10 (62.5)</td>
<td>blue-green</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>429</td>
<td>422 (99)</td>
<td>pink</td>
<td>6 (37.5)</td>
<td>beige</td>
</tr>
<tr>
<td><em>Enterococcus spp.</em></td>
<td>17</td>
<td>17 (100)</td>
<td>pink</td>
<td>25 (15)</td>
<td>pink to brown, colonies to beige</td>
</tr>
<tr>
<td><em>Enterococcus spp.</em></td>
<td>113</td>
<td>213 (100)</td>
<td>metallic blue with or without pink halo</td>
<td>17 (100)</td>
<td>blue-green</td>
</tr>
<tr>
<td><em>Hafnia alvi</em></td>
<td>3</td>
<td>2 (66.7)</td>
<td>blue to turquoise, small</td>
<td>213 (100)</td>
<td>blue to turquoise, small</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>96</td>
<td>96 (100)</td>
<td>beige</td>
<td>96 (100)</td>
<td>blue-green</td>
</tr>
<tr>
<td><em>Morganella morgani</em></td>
<td>7</td>
<td>7 (100)</td>
<td>metallic blue with or without pink halo</td>
<td>7 (100)</td>
<td>blue-green</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>61</td>
<td>61 (100)</td>
<td>beige with brown halo</td>
<td>61 (100)</td>
<td>beige with brown halo</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5</td>
<td>5 (100)</td>
<td>beige</td>
<td>5 (100)</td>
<td>beige</td>
</tr>
<tr>
<td><em>Providencia spp.</em></td>
<td>16</td>
<td>16 (100)</td>
<td>blue with brown halo</td>
<td>16 (100)</td>
<td>beige with brown halo</td>
</tr>
<tr>
<td><em>Pseudomonas spp.</em></td>
<td>57</td>
<td>53 (93)</td>
<td>transparent, yellow to green, normal edge, diffused</td>
<td>35 (65.2)</td>
<td>beige with brown halo, transparent to green, normal edge, diffused</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>6</td>
<td>6 (100)</td>
<td>blue</td>
<td>22 (38.5)</td>
<td>blue with or without green halo</td>
</tr>
<tr>
<td><em>Sphingobacterium saprophyticum</em></td>
<td>1</td>
<td>1 (100)</td>
<td>beige</td>
<td>1 (100)</td>
<td>beige</td>
</tr>
<tr>
<td><em>Sphingomonas spp.</em></td>
<td>36</td>
<td>36 (100)</td>
<td>light blue, pin-like</td>
<td>32 (88.9)</td>
<td>white, light blue, pin-like</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6</td>
<td>6 (100)</td>
<td>pink, opaque</td>
<td>4 (11.1)</td>
<td>blue-green</td>
</tr>
<tr>
<td><em>Staphylococcus spp.</em></td>
<td>19</td>
<td>19 (100)</td>
<td>golden, opaque, white, pink</td>
<td>6 (100)</td>
<td>white</td>
</tr>
</tbody>
</table>

**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 been 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. Uncultivable, 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 per-
formances of commercially available CRH and CPS chro-
monicogenic media were evaluated and compared with
one another as well as with other standard media em-
ployed 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 uri-
nary tract pathogens, to support the growth of all the iso-
lates, and to evaluate their growth quantitatively; up to
now, the combined use of two conventional media has been
required to achieve detection, growth, and quantita-
tive assessments. Unlike blood agar, these chromogenic
media inhibit or significantly decrease the swarming of
Proteus, Esherichia coli, Klebsiella, and Eiecrosis strains,
due to the production of a bactericidal agent and a correct
detection of the colonist to be obtained.

The most common enterococci that produce uri-
nary tract infections are gram-negative bacteria, which
belong to the Enterobacteriaceae family. CRH and CPS
media used different chromogenic substrates to detect
specific bacterial enzymes, which allow identification of
Esherichia coli. Presumptive identification of Escheri-
chia coli strains, which accounted for 43% of the clini-
cally significant isolates in our study, was better on CRH
medium (99% identified), which detects β-galactosidase
activity, than on CPS medium (90% identified), which
detects β-glucosidase enzyme activity. This dif-
fERENCE is significantly significant (P<0.01, chi-square
test). The superior sensitivity of β-galactosidase enzyme
activity in comparison with β-glucosidase enzyme ac-
tivity for identification of Escherichia coli has recently
been reported [6, 14].

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

On the chromogenic media, a correct identification of
all pink-to-bordeaux colonies must be confirmed by an
indole test, since some strains of Salmonella spp., Cit-
trullus 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 diversum also yield pink-to-
red colonies on CRH medium and produce a positive in-
dole test, leading to misidentification as Esherichia
coli strains (Table 2).

Enterococci were correctly identified on both chro-
monicogenic media at the genus level. The two most com-
non enterococcal species, Enterococcus faecalis and En-
terococcus 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
osteomucous infections and most often were present in
cystoureter-associated cystitis.

All of the Proteus mirabilis isolates were chromogen-
ically distinct on both chromogenic media, which yield-
ed the same rate of detection for all isolates of the Pro-
tes-Morganella-Provision 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 Esherichia coli or Klebsiella pneumoniae var.
oxynassensus. The possibility that the metabolism of the
crypto-organisms can vary in the medium was suggested previ-
ously [8, 14], and for correct identification, it is recom-
manded 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 meta-
lic-blue and blue-green colonies on CRH and CPS chro-
monicogenic media, but further biochemical tests were
needed to differentiate them to a species level.

The Pseudomonas spp. strains were differentiated and
distinguished chromogenically, with varying degree 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 CRH media enabled correct identification of the
Staphylococcus saprophyticus isolates, which pro-
duced typical pink-colored colonies, as confirmed by
previous reports [6, 11]. In our study, two Staphylococcus
simulans isolates also produced pink-colored 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 chromo-
genic media enabled easy differentiation of the isolates
in mixed cultures.

The well-distincted colonies produced by some of
the species enabled rapid presumptive identification of
the main bacteria responsible for urinary tract infections;
con-
sequently, a few additional confirmatory tests are required.
In this study, Esherichia 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 sus-
cceptibility tests carried out by using samples of the col-
onies taken directly from the standard medium (blood
agar) at a small number of minor discrepancies in zone
inhibition sizes was observed; these variations in category
occurred with antimicrobial agents that had zone-size
difference 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 suscept-
ceptibility 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 advan-
tageous, as it enables a single medium to be used in the screening of clinical urine samples.

One additional factor to consider is the time and cost required for screening urine cultures with multi-
ple types of media. We have estimated our costs at U.S. dollars (U.S.S. 5.5) for the conventional dual-plating method (CLID) and TSA with 5% sheep blood or TSA with 5% sheep blood and MacConkey agar, U.S.S.30.55 for CHROM agar, and U.S.S.04.44 for CPS medium. Fur-
thermore, it seems clear that the application of a single medium (CHROM or CPS medium) resulted in a 30% reduc-
tion in the time required for the processing of urine spec-
imens and for the examination of culture.

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

Overall, CHROM medium achieved a diagnostic yield that was slightly superior to that of the CPS medium. CHROM medium produced a more intense colouration of the colo-
NIST and performed better in the presumptive identifica-
tion of Escherichia coli and Staphylococcus aureus in 18.548 strains and in differentiating between Enterobacteriaceae and Pseudomonas aeruginosa strains, generally requiring a shorter incubation time than conventional tests for correct identification of the species (249 APID tests with CHROM medium and 256 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 isolate; (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 swelling of Proteus and other motile bacteria; and (vi) enable susceptibility testing to be performed without the need for further subcultures. The use of the chromogenic media described here should therefore significantly reduce the daily workload and decrease both the time ex-

depended and the related costs in favour of better perform-
ance and a more efficient use of available resources.

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