

Colonial morphology of *Escherichia coli*: impact of detection in clinical specimens

Lucia Barcella, Angelo Pasquale Barbaro, Santa Beatrice Rogolino

Department of Microbiology, Bianchi Melacrino Morelli Hospital, Reggio Calabria, Italy

Summary

As part of bacteriological identification, the macroscopic observation of the colonies on a culture medium is a critical step as it allows the clinical microbiologist to conduct a primary screening of microorganisms and in some cases even to identify them. This study shows how the macroscopic observation of the colonies, in particular of the colonies of *Escherichia coli* isolated on MacConkey agar from various clinical specimens, allowed to identify 5 classes of morphological variants of the colonies or *colonial morphology*, thanks to the knowledge and the experience gained by the clinical microbiologist.

Introduction

As part of bacteriological identification, the macroscopic observation of the colonies on a culture medium is a critical step, as it allows the clinical microbiologist to conduct a primary screening of the microorganisms.

It provides for a two-stage approach: i) a presumptive or preliminary

identification, based on macroscopic and microscopic observation of the colonies; ii) the confirmation of the results of the presumptive identification through secondary tests (biochemical, serological, molecular tests), such as those provided by commercial identification systems (definitive identification).

This study shows how the macroscopic observation of the colonies, in particular those of *Escherichia coli* grown on MacConkey agar from various clinical specimens, allowed to identify five classes of morphological colony variants or *colonial morphology* (CM), thanks to the knowledge and the experience gained by the clinical microbiologist (also Rhodes K. S., in 1966, defined them this way) (4).

Materials and Methods

The study, aiming to evaluate the incidence and the antibiotic-resistance profiles of the CM of 885 *E. coli* strains collected from different clinical samples of 712 patients, was carried out between October 2014 and May 2015.

A total of 885 *E. coli* strains was studied divided as follows: i) 645 were from inpatients, obtained from faecal material (n=285), urine (n=270), respiratory specimens (n=20), effusions (n=16), venous catheters (n=13), blood cultures (n=13), swabs and other samples (n=28); ii) 240 were from outpatients, obtained from urine (n=128), faeces (n=97), swabs and other samples (n=10), respiratory specimens (n=5).

The *E. coli* strains, at first were isolated in pure culture on MacConkey Agar and then, after 24 hours of incubation at 37°C, subjected to identification and antibiogram analysis by the semi-automated System Vitek2 (bioMérieux, Marcy l'Etoile, France).

Results and Discussion

Between October 2014 and June 2015, 885 strains of *E. coli* have been studied, 645 (73%) being from hospitalized patients and 240 (27%) from outpatients.

The results of this work have been achieved from the observation of the macroscopic colonies of *E. coli* on MacConkey agar after no more than 24 hours of incubation at 37°C in fact, as already Hasman (2) in 1999 noted, over this time the observed CM could be converted into one another.

Most of the collected *E. coli* isolates (n=835, 94%) showed bright pink colonies on MacConkey agar, while 50 strains (6%) produced yellow-amber colonies (Figure 1).

The macroscopic observation of *E. coli* strains grown on agar MacConkey allowed us to identify five main types of CM. Depending on the macroscopic aspect of the colonies, these CM series have been divided into five classes, as follows: CM I or domed (representing the

Correspondence: Lucia Barcella, Department of Microbiology, Bianchi Melacrino Morelli Hospital, via Sbarre Inferiori 417, 89100 Reggio Calabria, Italy.

Tel.: +39.340.9418543.

E-mail: luciabarcella@gmail.com

Key words: *Escherichia coli*; colonial morphology; macroscopic expression; MacConkey agar.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 17 November 2015.

Revision received: 1 February 2016.

Accepted for publication: 1 February 2016.

©Copyright L. Barcella et al., 2016

Licensee PAGEPress, Italy

Microbiologia Medica 2016; 31:5636

doi:10.4081/mm.2016.5636

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

typical morphology of *E. coli* colonies); CM II or umbilicated; CM III or flared; CM IV or cercinate; CM V or not fermenting lactose.

The percentages identified for each single morphology are reported in Table 1.

The class CM I represents 28% of the isolated *E. coli* strains (n=250) and it shows a dome-shaped appearance, firm, raised and opaque (Figure 2a). The class CM II represents 32% of the isolated strains (n=283) and shows a similar dome shaped but in the middle presents a slight depression and inside this, middle located, has a little bump point (Figure 2b). The class CM III represents 27% of isolated strains (n=243), appears flat and flared, often with uneven edges, with reduced thickness and larger than the classes CM I and CM II (Figure 2c). The class CM IV, representing 7% (n=59) isolated, is similar to the class CM II but shows significantly raised edges, a deeper central depression and a more pronounced central core (Figure 2d). These four classes appear bright pink colour on MacConkey agar, suggestive of bacterium ability to use the lactose present in the culture medium.

The class CM V is similar to the CM III class in dimensions and morphological characteristics but appears yellow-amber on MacConkey agar (Figure 3), a clear sign of the inability to use lactose; this class is the rarest, as it is identified only in 6% of the isolates (n=50).

The CM II could be defined as the *dominant CM*, because it's the more frequently identified among the *E. coli* strains evaluated in this study; the CM I and the CM III also represent a large percentage of the isolates, but they appeared in a lower proportion than the CM II. The CM IV and V CM are shown only in a smaller group of isolates.

It is interesting to note that a relevant proportion of the clinical *E. coli* isolates shown the coexistence of two or more different classes of

E. coli CM: of 382 examined faecal material samples, 54 (14%) showed different class of CM. In 82% of these cases the CM III class has been the most frequently identified.

No significant differences in CM classes were identified among *E. coli* isolates obtained from in- and outpatients as reported in Table 2 below.

The biochemical profiles of the 885 *E. coli* strains isolated, regardless of the class of CM, was obtained by Vitek2 System through the use of the GN Card (Gram Negative Card, bioMérieux). The percentages of positivity to the various biochemical tests relating to the five different classes of *E. coli* CM are reported in Table 3.

The five classes of CM showed coherent biochemical profiles (Table 3). The only relevant finding was the 100% positivity identified in 100% of the CM V isolates for the so called ELLM biochemical test, which, using Ellman's reagent (DTNB or 5,5'-dithio-bis [2- nitroben-

Table 1. Percentage of isolation for the five *E. coli* colonial morphology (CM) classes.

<i>E. coli</i> CM	N. samples	% isolation
I	250	28
II	283	32
III	243	27
IV	59	7
V	50	6
Total	885	100



Figure 1. *E. coli* pink colonies and yellow-amber colonies.

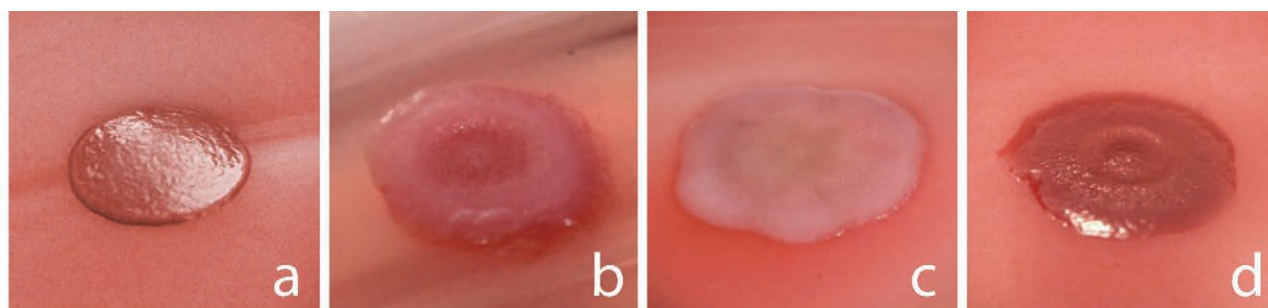


Figure 2. *E. coli* colonial morphology (CM): CM I (a), CM II (b), CM III (c) and CM IV (d) classes.

zoic acid]), quantifies the thiols that are molecules derived from the metabolism of methionine. The remaining CM classes showed a much lower percentage of strains positive to the ELLM test, ranging from 1.1 (CM II) to 5.2% (CM IV).

Data shown in Table 3 also indicate that 20% of the strains in the class CM V (n=10) are lacking Beta-Galactosidase given their negativity to the BGAL test.

The antibiotic susceptibility profiles of the 885 *E. coli* strains studied, were obtained by using the automated System Vitek2 with the Cards type 202/204 (bioMérieux); the results (in term of % sensitivity to each individual antimicrobial evaluated) are summarized in Figure 4.

Table 4 reports the detail about % sensitivity of the *E. coli* isolated divided by their CM class.

Overall, there were no significant differences of antibiotic susceptibility tested between the five classes of CM. Most of the isolates, as expected and according to the data from literature, are resistant to ciprofloxacin and ampicillin. 29.5% of the studied bacteria were also resistant to trimethoprim/sulfamethoxazole, while the resistance to -

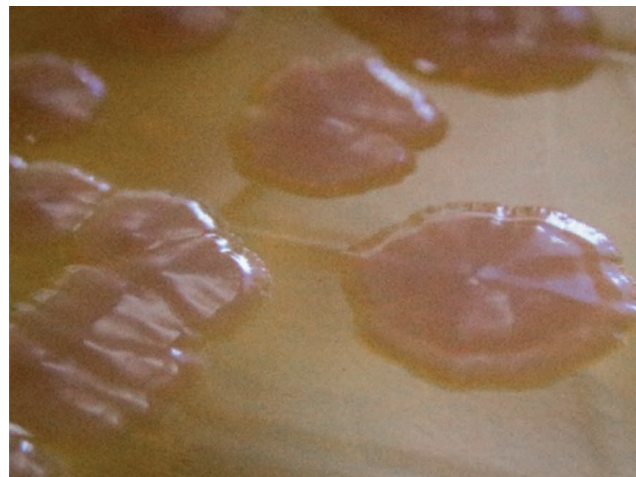


Figure 3. *E. coli* colonial morphology V class.

Table 2. Percentage of isolation of the five *E. coli* colonial morphology (CM) classes from inpatients and outpatients.

Population	CM I		CM II		CM III		CM IV		CM V		Total
	N.	%	N.	%	N.	%	N.	%	N.	%	
Hospitalised	200	31	208	32.3	168	26	39	6	30	4.7	645
Outpatients	50	21.1	75	31.3	75	31.3	20	8.3	20	8.3	240

Table 3. Percentage of positivity to biochemical tests of the five *E. coli* colonial morphology (CM) classes.

Biochemical tests	CM I	CM II	CM III	CM IV	CM V	Biochemical tests	CM I	CM II	CM III	CM IV	CM V
APPA	0	0	0	0	0	IARI	0	0	0	0	0
H2S	0.9	0	1.3	0	0	dGLU	99.1	100	99.3	94.7	98
BGLU	0	0	0	0	0	dMNE	99.1	100	99.3	94.7	100
ProA	30	17.2	27.3	31.6	14	TyrA	83.6	77.4	90	68.4	68
SAC	56.4	68.9	55.3	79	50	CIT	0.9	0	0	0.7	0
ILATk	49.1	23.6	40.6	31.6	32	NAGA	0	0	0	0	0
GlyA	0.9	0	4	5.2	2	IHISa	0	0	0	0	0
O129R	76.4	72	72.6	52.6	68	ELLM	2.7	1.1	4	5.2	100
ADO	6.4	2.1	5.3	0	0.8	dCEL	0	0	0	0	0
BNAG	0	0	0	0	0	GGT	3.6	0	3.3	0	2
dMAL	96.4	96.8	98	100	98	BXYL	0	0	0	0	0
LIP	0	0	0	0	0	URE	2.7	0	1.3	10.5	2
dTAG	29.1	32.3	21.3	73.7	16	MNT	0	0	0	0	0
AGLU	0	0	0	0	0	AGAL	80.9	86	89.3	78.9	84
ODC	62.7	78.5	50	89.5	70	CMT	98.2	97.8	98.6	100	94
GGAA	0	0	0	0	0	ILATa	1.8	1.1	2.7	0	0
PyrA	0	0	0	0	0	BGAL	100	100	100	100	80
AGLTp	0	0	0	0	0	OFF	95.4	98.9	98	94.7	84
dMAN	99.1	98.9	99.3	94.7	94	BAlap	0	0	0	0	0
PLE	0	0	0.7	0	0	dSOR	97.3	95.7	97.3	100	96
dTRE	99.1	98.9	100	94.7	96	5KG	22.7	38.7	35.4	31.6	24
SUCT	93.6	82.8	88	84.21	54	PHOS	1.8	1.1	6.7	5.3	6
LDC	91.8	94.6	87.3	89.5	96	BGUR	84.5	89.2	85.3	84.2	84
IMLTa	3.6	1	2.7	0	0						

lactamines with Beta-Lactamases inhibitors ranged between 8 and 20%. Furthermore gentamicin was the less effective among the aminoglycosides. Only 17.6% of *E. coli* strains (n=156) were ES L (Extended-Spectrum Beta-lactamases) producer.

Conclusions

The possibility to distinguish different *E. coli* CM, was already described by Rhodes in 1966 (4) on Tergitol-7 Agar and this has been confirmed in this study, thus highlighting the large versatility of macroscopic expression of these bacteria (7).

Considering that CM II type represents the most frequently identified CM among the *E. coli* strains studied, we could probably speculate that the classes less frequent of CMs likely differentiated more recently than CM II, and this could justify their low rate of identification. Based also on data by Drucker and Whittaker (1), we can assume that the different classes of CM are not dependent on the metabolic profiles, that apparently differ only in the class CM V (characterized by the inability to ferment the lactose and by a stronger metabolism of methionine), but this could rather depend on the different arrangement that individual bacterial cells implement during the process of colony development (6).

In our opinion, the characterization of the features of different bacterial isolates should not be based only on the study of biochemical profiles and antibiotic susceptibility. Standard procedures should be sup-

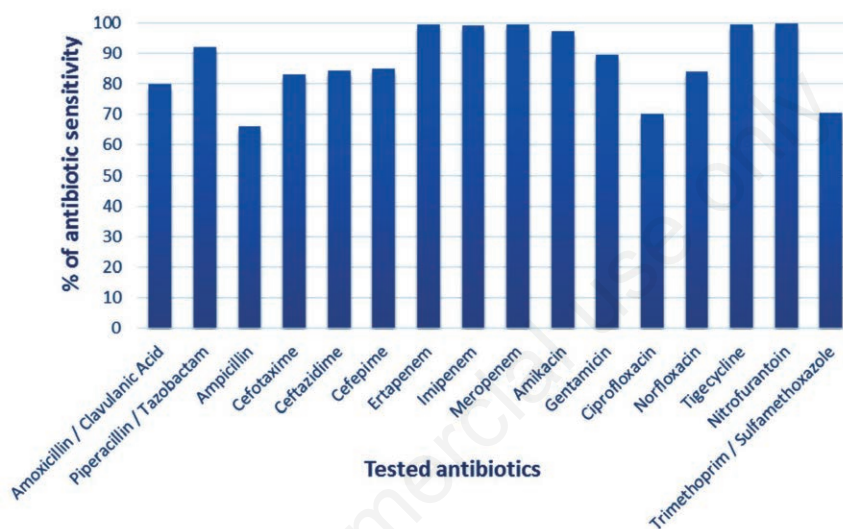


Figure 4. Percentage of the total antibiotic susceptibility of *E. coli* isolated strains.

Table 4. Percentages of antibiotic sensitivity of the five *E. coli* colonial morphology (CM) classes.

Antibiotics	Tot <i>E. coli</i>	% of sensitivity against tested antibiotics				
		CM I	CM II	CM III	CM IV	CM V
Amoxicillin/Clavulanic Acid	80	74	87.3	79.8	76.3	82
Piperacillin/Tazobactam	92	89.2	94.3	92.2	91.5	92
Ampicillin	66.2	62	74.6	63.8	61	58
Cefotaxime	83	75.6	90.1	85.2	72.9	82
Ceftazidime	84.5	77.6	89.7	87.2	76.3	86
Cefepime	85.1	79.2	90.1	87.2	78	84
Ertapenem	99.4	99.2	100	98.8	100	100
Imipenem	99.2	98.8	100	98.3	100	100
Meropenem	99.5	99.6	99.3	99.6	100	100
Amikacin	97.3	95.6	98.6	96.7	98.3	100
Gentamicin	89.6	86.8	92.2	91.8	88.13	80
Ciprofloxacin	70.3	60.4	82.3	67.5	64.4	72
Norfloxacin	84.1	84	89.7	81.1	83	68
Tigecycline	99.3	99.2	100	98.8	100	98
Nitrofurantoin	99.7	99.6	100	99.6	100	98
Trimethoprim/Sulfamethoxazole	70.5	63.2	74.6	72.8	74.6	68

ported and complemented by the observation of the morphology and colonies disposition. This *old new way* to discriminate different *E.coli* strains could be the starting point for a deeper investigation the link between the macroscopic expression and microscopic features of bacteria (5). This work could thus be an innovative stimulus for Clinical Microbiologists to better observe and study the microorganisms not only considering their possible (pathogenic) effects on the human host, but also as a guide to understand a world still largely unknown.

References

1. Drucker DB, Whittaker DK. Microstructure of colonies of rod-shaped bacteria. J Bacteriol 1971;108:515-25.
2. Hasman H, Schembri MA, Klemm P. Antigen 43 and type 1 fimbriae determine colony morphology of *Escherichia coli* K-12. J Bacteriol 2000;182:1089-95.
3. Murray P, Tenover FC, Pfaller MA, eds. Manual of clinical microbiology. 6th Edition. Washington: ASM Press; 2009.
4. Rhodes KS. Colonial morphology of *Escherichia coli* on tergitol-7 medium. Appl Microbiol 1966;14:152-5.
5. Shapiro JA. Organization of developing *Escherichia coli* colonies viewed by scanning electron microscopy. J Bacteriol 1987;169:142-56.
6. Su PT, Liao CT, Roan JR, et al. Bacterial colony from two-dimensional division to three-dimensional development. PLoS One 2012; 7:e48098.
7. Zinnah MA, Bari MR, Islam MT, et al. Characterization of *Escherichia coli* isolated from samples of different biological and environmental sources. Bangladesh J Vet Med 2007;5:25-32.

Non-commercial use only