L'esperienza del Laboratorio di Microbiologia nel paziente adulto

SESSIONE 14

LO STUDIO DEL MICROBIOTA: IL RUOLO DELL'AMCLI

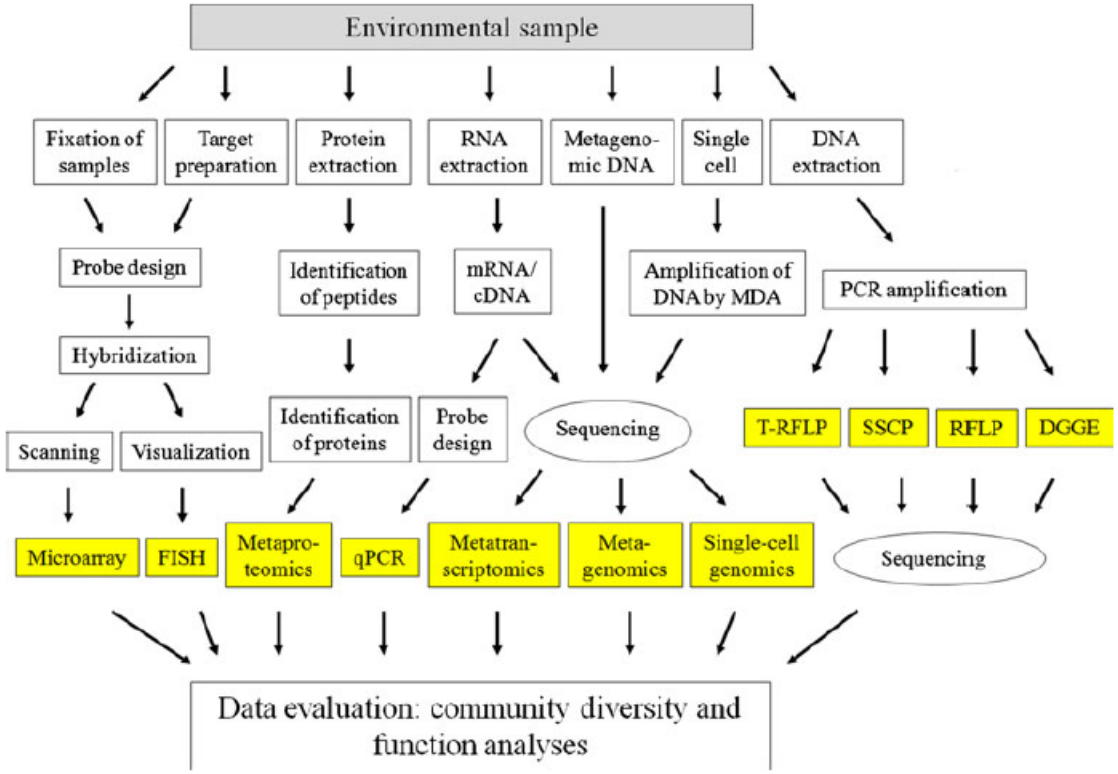
Luca Masucci

Appl Microbiol Biotechnol (2012) 93:993–1003
DOI 10.1007/s00253-011-3800-7

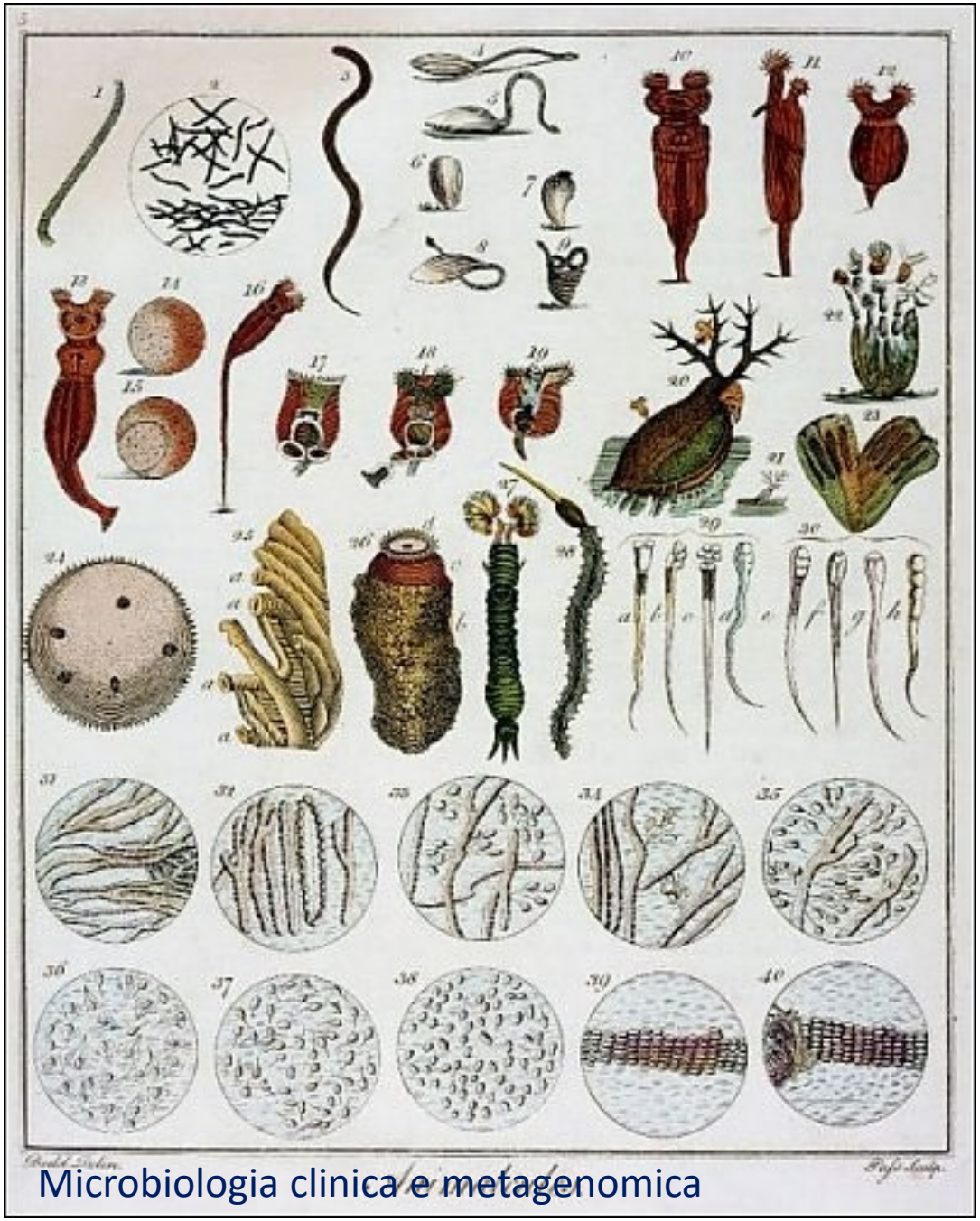
MINI-REVIEW

Culture-independent methods for studying environmental microorganisms: methods, application, and perspective

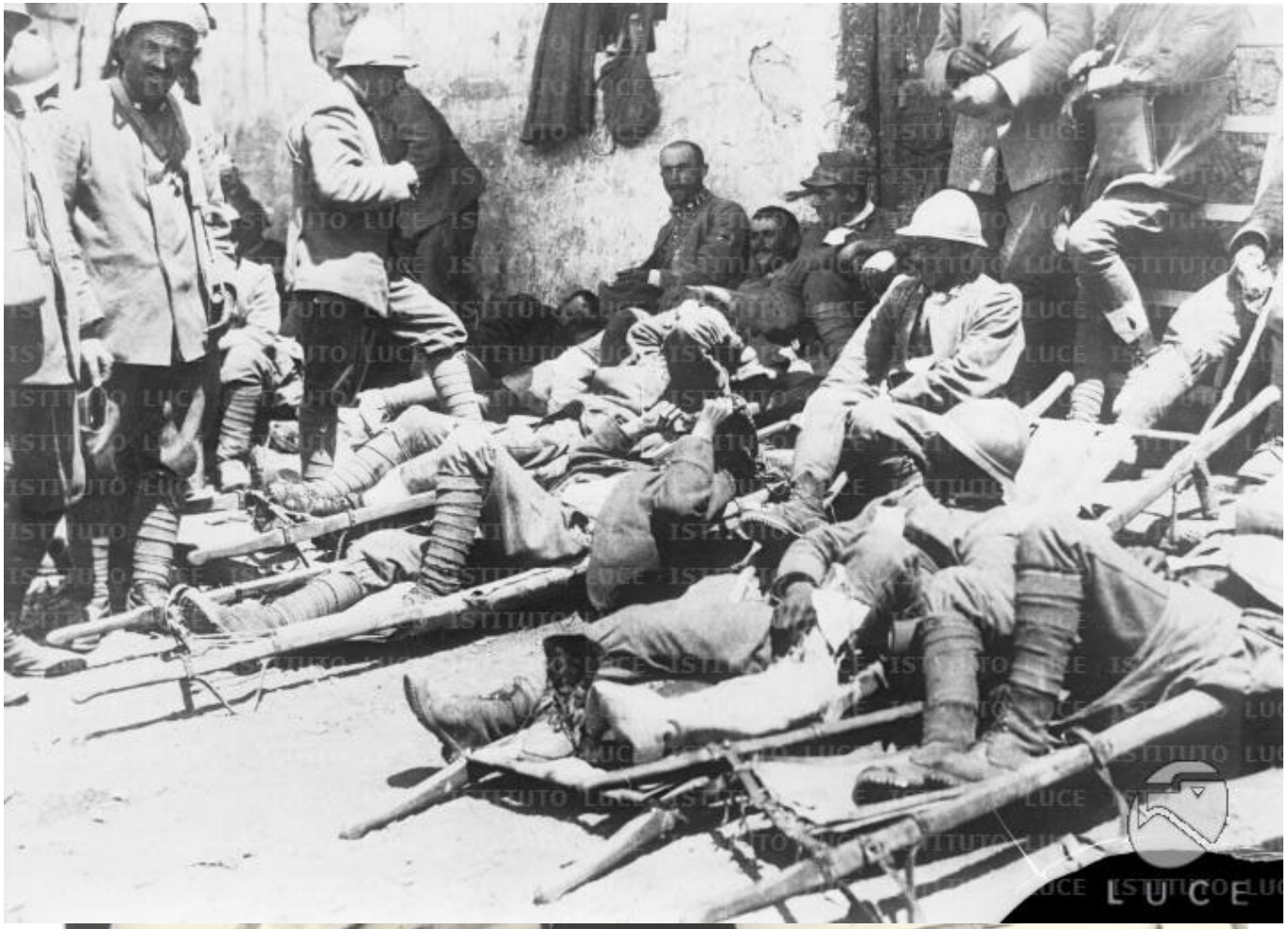
Can Su · Liping Lei · Yanqing Duan · Ke-Qin Zhang · Jinkui Yang



Antony van Leeuwenhoek



Microbiologia clinica e metagenomica


LUCE

BENGTSSON E

FATIGUE AFTER PENICILLIN ADMINISTRATION

Svenska läkartidningen 1951 Oct 5;48(40):2334-8.

FURBERG C, RINGQVIST T.

EFFECT OF PENICILLIN ON THE PHYSICAL CAPACITY FOR WORK AND ON THE EXPERIENCE OF FATIGUE DURING WORK

Sven Lakartidn. 1963 Sep 11;60:2650-5.

Microflora

Skin	Dry, acidic conditions, <37°C	Limit bacterial growth
	Sloughing cells	Remove bacteria
	Resident microflora	Compete for colonization sites
Hair follicles, sweat glands	Lysozyme, toxic lipids	Kill bacteria
Beneath skin surface	SALT	Kill bacteria; sample antigens on skin surface
Mucosal surface	Mucin layer	Physical barrier, trap bacteria
Mucin layer	Lysozyme	Digest bacterial peptidoglycan
	sIgA	Prevent bacterial attachment to mucosal cells, help to trap bacteria in mucin
	Lactoferrin	Bind iron, prevent bacterial growth
	Lactoperoxidase	Kill bacteria by generating toxic superoxide radicals
Mucous membrane	Sloughing cells	Remove adherent bacteria
	Tight junctions	Prevent bacteria from invading between mucosal cells
Beneath mucosal membrane	MALT	Produce sIgA; phagocytic cells kill bacteria

TABELLA 23.2 Attività biochimiche e metaboliche della flora intestinale

Sintesi di vitamine	Prodotto: tiamina, riboflavina, piridossina, vitamine B_{12} e K
Produzione di gas	Prodotto: CO_2, CH_4, H_2
Produzione di sostanze odorose	Prodotto: H_2S, NH_3, amine, indolo, scatolo, acido butirrico
Produzione di acidi organici	Prodotto: acidi acetico, propionico e butirrico
Reazioni di glicosidazione	Enzima: β-glucoronidasi, α- e β-galattosidasi, α- e β-glucosidasi
Metabolismo degli steroidi (acidi biliari)	Processo: esterificazione, deidrossilazione, ossidazione, riduzione, inversione

netagenomica


Be-Total
Classico
Be-Total


MICROFLORA

Microbiota
definizione

Il microbiota umano consiste di microrganismi che esistono su, all'interno o in prossimità del corpo umano.

Archea, Batteri, virus, fagi, funghi

Microbioma
definizione

Il microbioma è l'insieme dell'ambiente e dei

microrganismi

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

New trends in *Clostridium difficile* virulence and pathogenesis

C. Denève[a], C. Janoir[a], I. Poilane[b], C. Fantinato[b], A. Collignon[a,b,*]

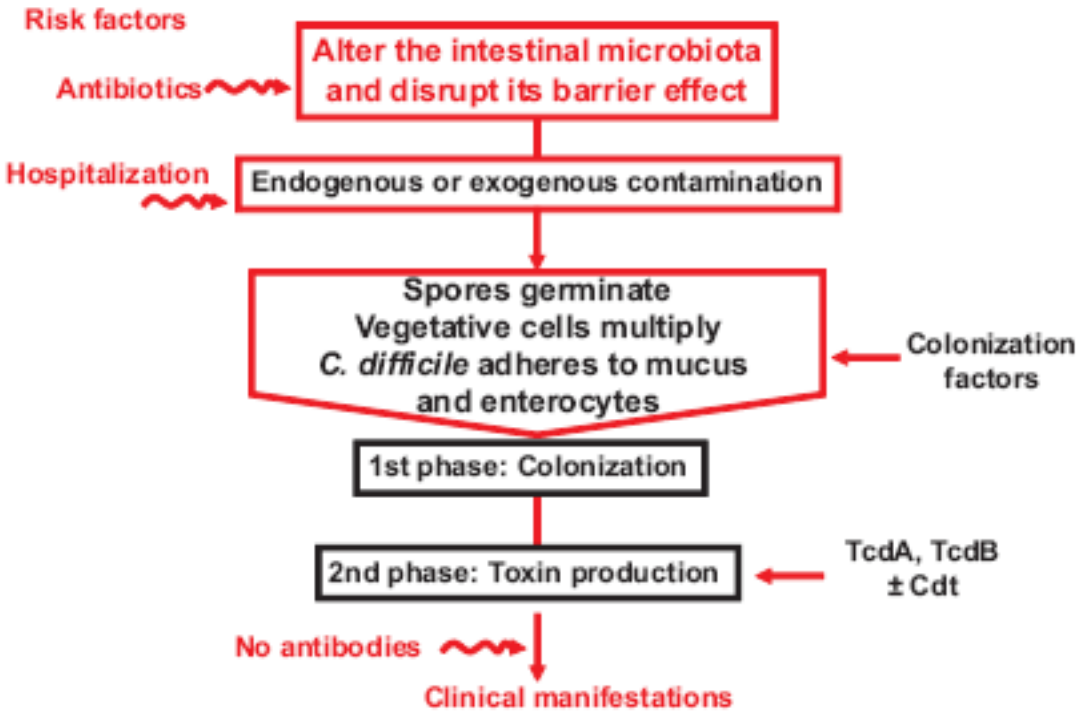
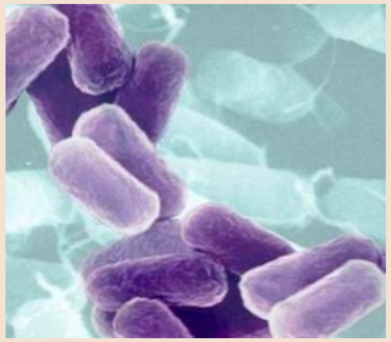


Fig. 1. Pathogenesis of *Clostridium difficile*.

25 giu 2013

MEDICINA E RICERCA

Al Gemelli di Roma il primo trapianto nel Lazio di flora batterica intestinale



Trapiantare il microbiota - la flora batterica intestinale - potrebbe rivelarsi la nuova frontiera per combattere molte malattie, tra cui il diabete e l'obesità. A sondare questa possibilità è un gruppo di ricerca del Policlinico Agostino Gemelli di Roma, che ha eseguito con successo il primo innesto di questo tipo nel Lazio (e il secondo in Italia) da un soggetto sano a un paziente affetto da una forma di diarrea legata a un'infezione da clostridium difficile resistente agli antibiotici.

Il trapianto - effettuato da un'équipe coordinata da Antonio Gasbarrini, responsabile dell'Unità operativa complessa di medicina interna e gastroenterologia del Gemelli - è stato effettuato attraverso una colonscopia eseguita da Giovanni Cammarota della Uoc di gastroenterologia e il microbiota del donatore (un parente) è stato preparato e purificato da Luca Masucci nell'ambito di una collaborazione con l'Istituto di microbiologia dell'Università Cattolica diretto da Maurizio Sanguinetti.

Contents lists available at ScienceDirect

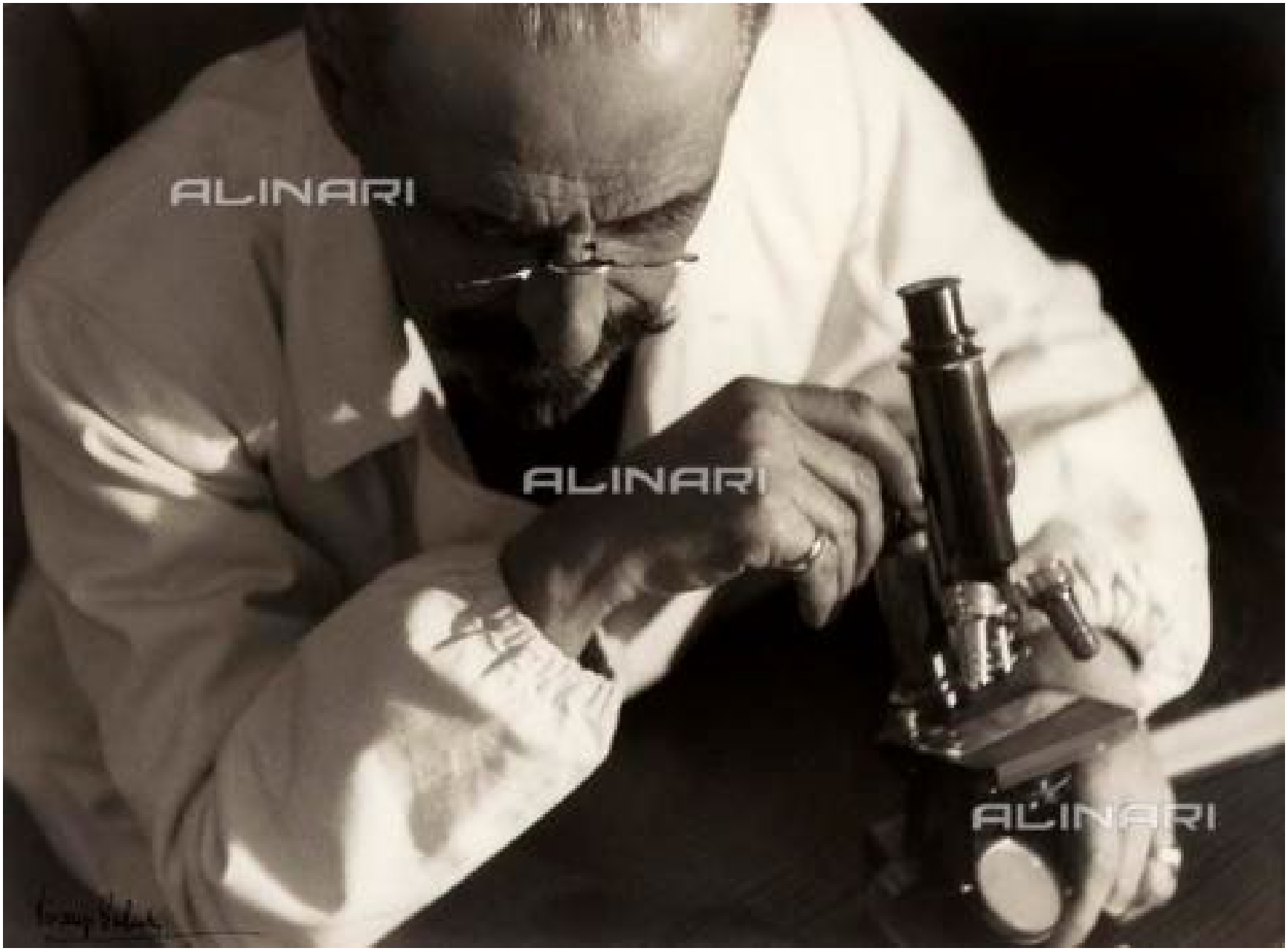
Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Faecal transplantation for *Clostridium difficile* infection. Three cases treated in Italy

Giovanni Cammarota*
Gianluca Ianiro
Antonio Gasbarrini
Department of Clinical Sciences, Division of Internal Medicine and Gastroenterology, A. Gemelli University Hospital, Rome, Italy

Luca Masucci
Maurizio Sanguinetti
Institute of Microbiology, A. Gemelli University Hospital, Rome, Italy


ALINARI
ALINARI
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ORIGINAL ARTICLE BACTERIOLOGY

Microbial culturomics: paradigm shift in the human gut microbiome study

J.-C. Lagier[1,*], F. Armougom[1,*], M. Million[1], P. Hugon[1], I. Pagnier[1], C. Robert[1], F. Bittar[1], G. Fournous[1], G. Gimenez[1], M. Maraninchi[2], J.-F. Trape[3], E. V. Koonin[4], B. La Scola[1] and D. Raoult[1]

1) Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, 2) Service de Nutrition, Maladies Métaboliques et Endocrinologie, UMR-INRA U1260, CHU de la Timone, Marseille, France, 3) IRD, UMR CNRS 7278-IRD 198, Route des Pères Maristes, Dakar, Sénégal and 4) National Centre for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA

nature microbiology

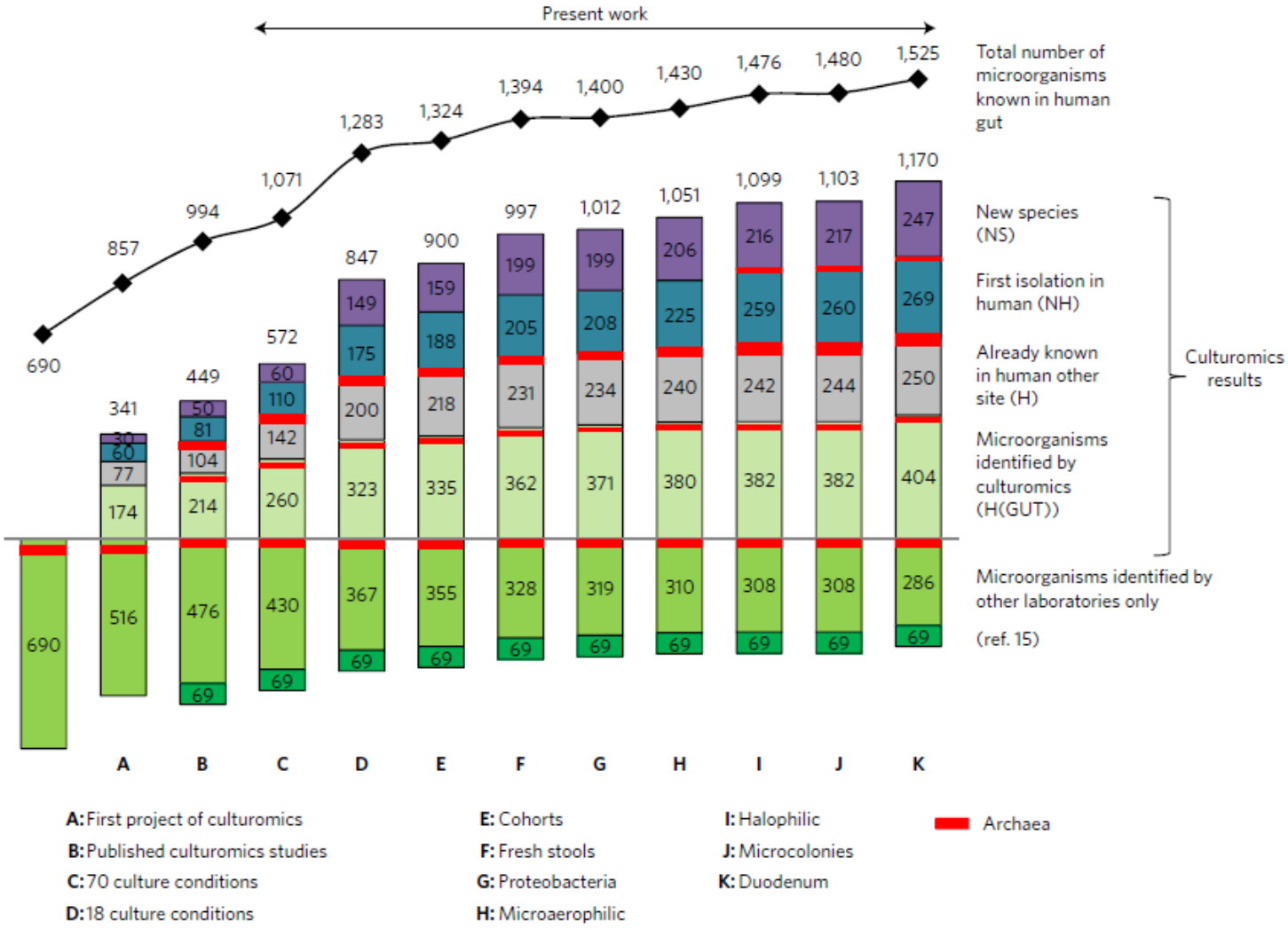
LETTERS

PUBLISHED: 7 NOVEMBER 2016 | ARTICLE NUMBER: 16203 | DOI: 10.1038/NMICROBIOL.2016.203

OPEN

Culture of previously uncultured members of the human gut microbiota by culturomics

Jean-Christophe Lagier[1], Saber Khelaifia[1], Maryam Tidjani Alou[1], Sokhna Ndongo[1], Niokhor Dione[1], Perrine Hugon[1], Aurelia Caputo[1], Frédéric Cadoret[1], Sory Ibrahima Traore[1], El Hadji Seck[1] Gregory Dubourg[1], Guil Sara Bellali[1], Dipankar B Davide Ricaboni[1], Melh Camille Valles[1], Donia M Esam Ibraheem Azhar[4], Felix Djossou[6], Véroniq Pierre-Edouard Fournie



nature microbiology

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OPEN

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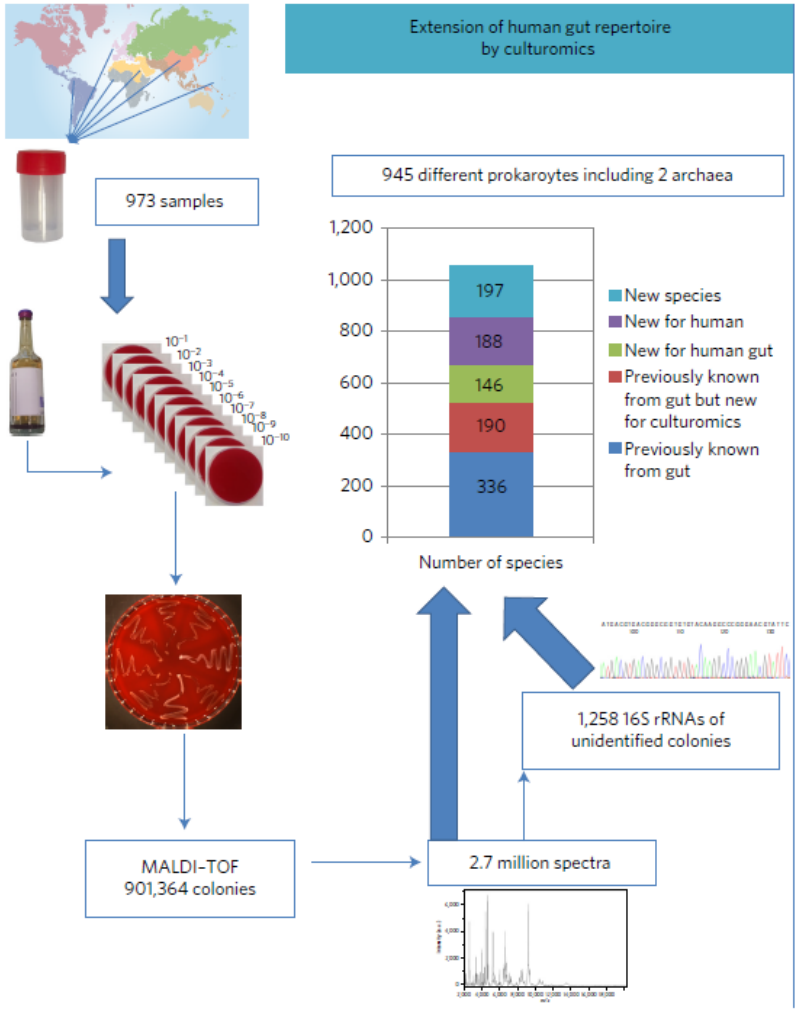
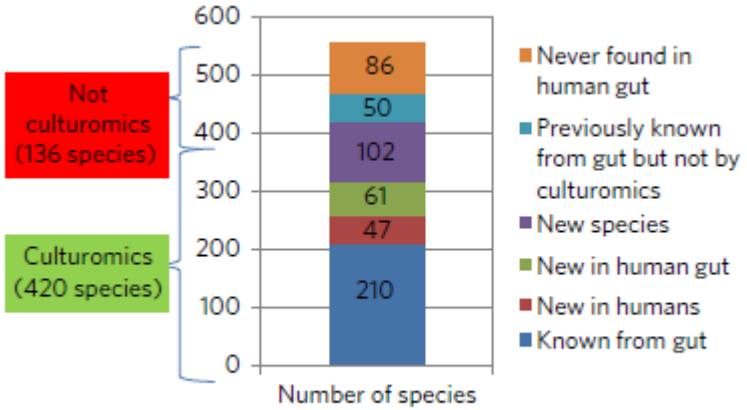
Davide Ricaboni[1],
Camille Valles[1], D
Esam Ibraheem A
Felix Djossou[6], V
Pierre-Edouard Fo





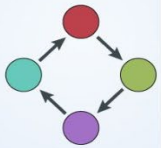
Culturing the human microbiota and culturomics

*Jean-Christophe Lagier[1], Grégory Dubourg[1], Matthieu Million[1], Frédéric Cadoret[2], Melhem Bilen[1,3], Florence Fenollar[4], Anthony Levasseur[1], Jean-Marc Rolain[1], Pierre-Edouard Fournier[4] and Didier Raoult[1]**

Strengths

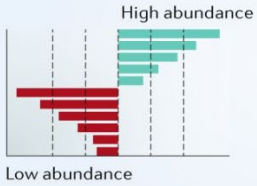
Inclusion of a large number of samples

Integrative workflows

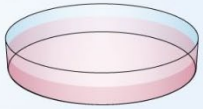


Time to result

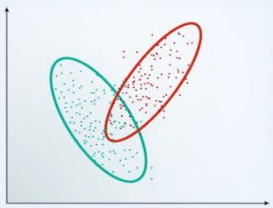
Detection of differentially abundant taxa



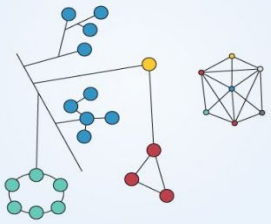
Detection of uncultured bacteria



Detection of microbial signatures and clustering

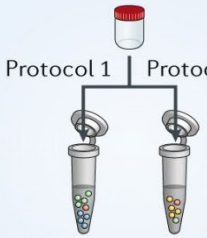


Functional analysis



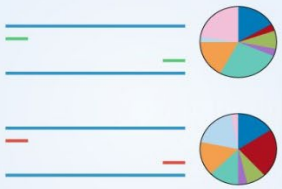
Weaknesses

Extraction bias



The bacterial diversity that is captured is a function of the extraction protocol

Primer bias



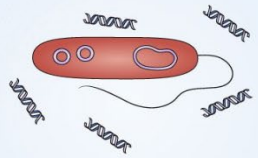
Species identification is dependent on the targeted hypervariable region

Bioinformatics biases

Variations in methodology

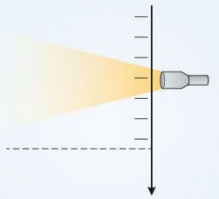
Discrimination between species is difficult owing to amplicon length

Viability bias



Cannot discriminate between live bacteria and transient DNA

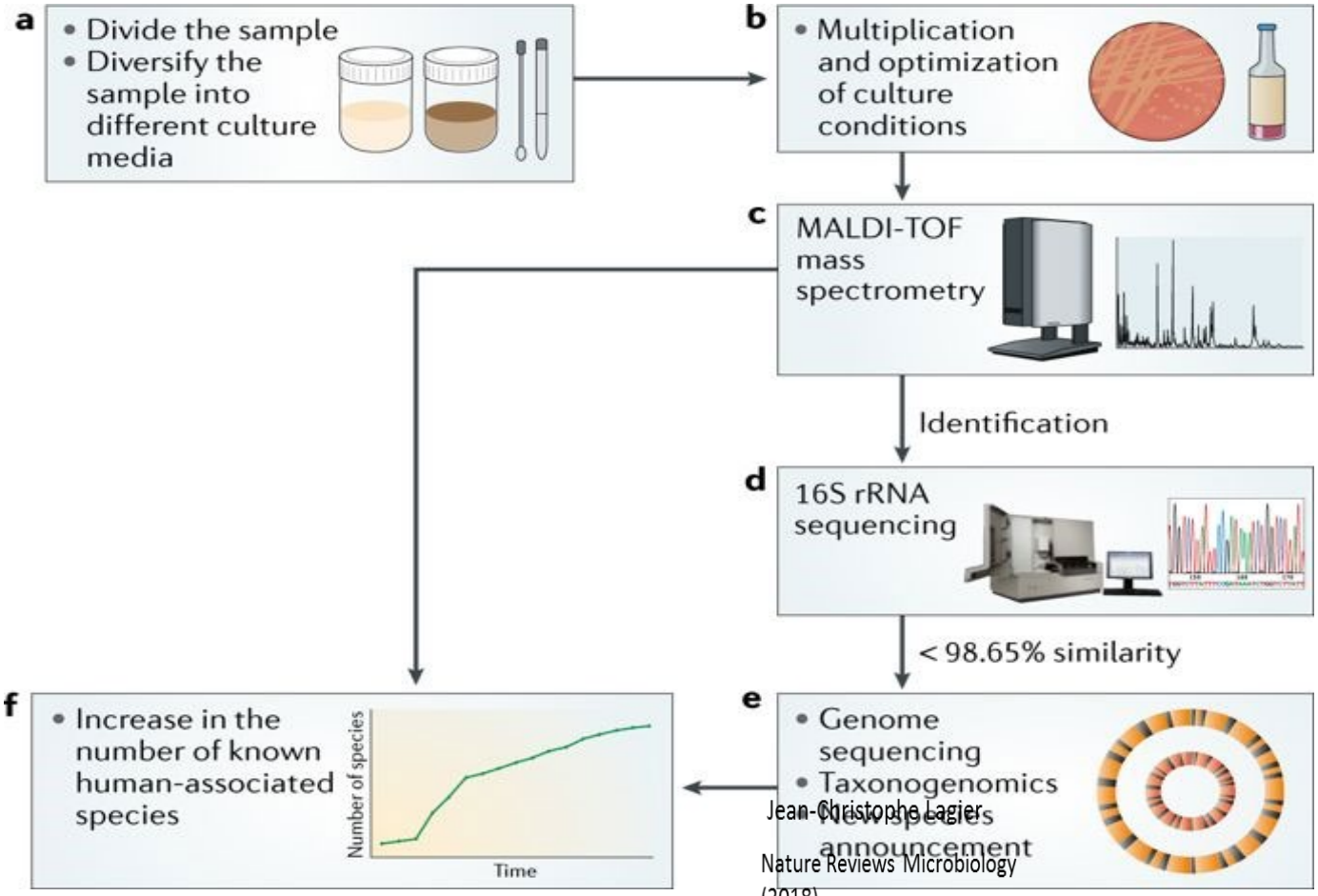
Depth bias



Minority populations are insufficiently detected

Culturing the human microbiota and culturomics

*Jean-Christophe Lagier[1], Grégory Dubourg[1], Matthieu Million[1], Frédéric Cadoret[2], Melhem Bilen[1,3], Florence Fenollar[4], Anthony Levasseur[1], Jean-Marc Rolain[1], Pierre-Edouard Fournier[4] and Didier Raoult[1]**



Jean-Christophe Lagier
Nature Reviews Microbiology (2018)

Microbiologia Medica 2017; volume 32:6510

Culturomics: bacterial species isolated in 3 healthy donors for faecal microbiota transplantation in *Clostridium difficile* infection

Luca Masucci,[1] Gianluca Quaranta,[1] Domenico Nagel,[1] Sandra Primus,[2] Lucio Romano,[1] Rosalia Graffeo,[1] Gianluca Ianiro,[3] Antonio Gasbarrini,[3] Giovanni Cammarota,[3] Maurizio Sanguinetti[1]

[1]Institute of Microbiology, Catholic University of the Sacred Heart, A. Gemelli Foundation, School of Medicine, Rome; [2]College for Healthcare Professions, Catholic University of the Sacred Heart, Claudiana, Bolzano; [3]Internal Medicine, Gastroenterology and Liver Unit, Catholic University of the Sacred Heart, School of Medicine, A. Gemelli Foundation, Rome, Italy

Table 1. Media and culture conditions.

1.	Pre-incubation in paediatric blood culture bottle with rumen fluid and then 5% sheep blood, Chocolate and McConkey agar under aerobic, aerobe with 2.5% CO_2 and microaerophilic conditions at 37°C
2.	Pre-incubation in anaerobic blood culture bottle with rumen fluid and then Columbia and Schaedler agar under anaerobic conditions at 37°C
3.	Pre-incubation in paediatric blood culture bottle with Brucella broth and then 5% sheep blood, Chocolate and McConkey agar under aerobic, aerobe with 2.5% CO_2 and microaerophilic conditions at 37°C agar under aerobic conditions at 37°C
4.	Pre-incubation in anaerobic blood culture bottle with Brucella broth and then Columbia and Schaedler agar under anaerobic conditions at 37°C
5.	Pre-incubation under aerobic conditions in BHI and then 5% sheep blood, Chocolate and McConkey agar under aerobic, aerobe with 2.5% CO_2 and microaerophilic conditions at 37°C agar under aerobic conditions at 37°C
6.	Pre-incubation in anaerobic blood culture bottle with stool filtered at 5 m and then Columbia and Schaedler agar under anaerobic conditions at 37°C
7.	Pre-incubation in paediatric culture bottle with stool filtered at 5 m and then 5% sheep blood under aerobic conditions at 37°C
8.	Pre-incubation in paediatric blood culture bottle with 5 mL BHI and then 5% sheep blood, Chocolate and McConkey agar under aerobic, aerobe with 2.5% CO_2 and microaerophilic conditions at 37°C agar under aerobic conditions at 37°C
9.	Pre-incubation in anaerobic blood culture bottle with 5 mL BHI and then Columbia and Schaedler agar under anaerobic conditions at 37°C

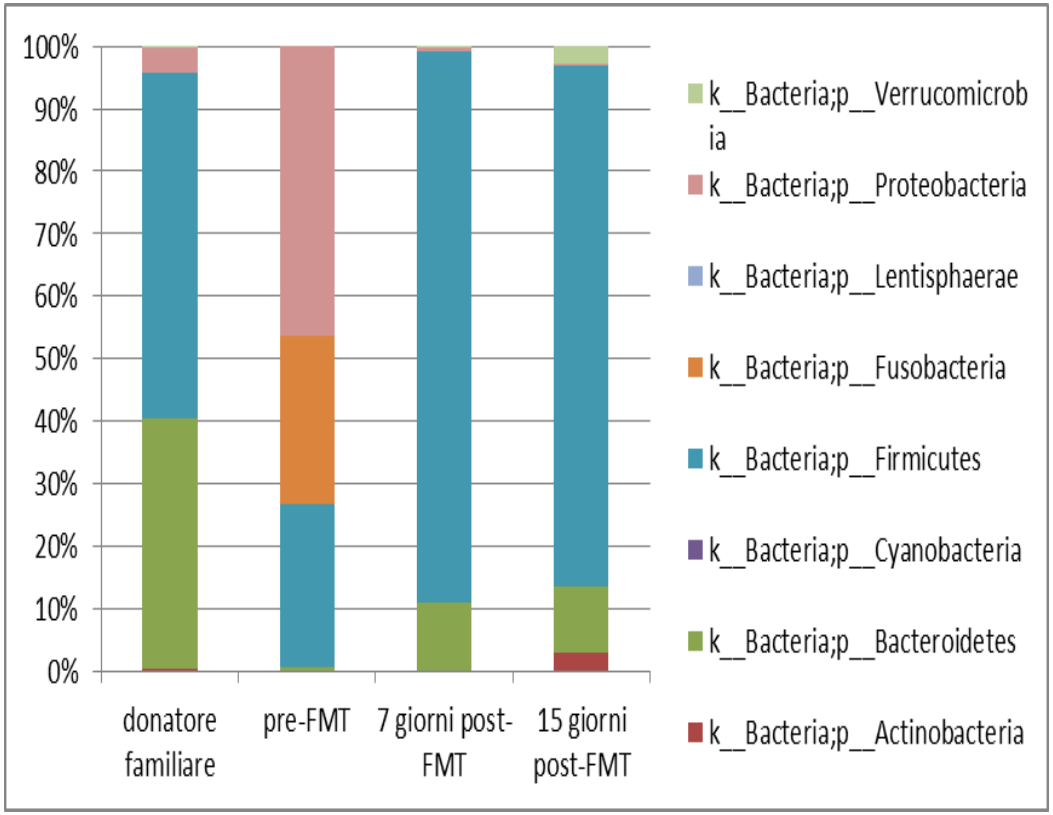
BATTERI ISOLATI					
			pre-trapianto	post trapianto 7gg	post trapianto 15gg
donatore	Acidaminococcus intestini	ricevente	Clostridium perfringens	Clostridium bifermentans	Acidamicoccus intestini
	Bacteroides fragilis		Clostridium ramosum	Clostridium scindens	Alistipes onderdonkii
	Bacteroides uniformis		Enterococcus casselliflavus	Enterococcus faecium	Bacteroides vulgatus
	Clostridium bifermentans		Enterococcus faecium	Enterococcus gallinarum	Clostridium baratii
	Clostridium clostridiforme		Enterococcus gallinarum	Enterococcus avium	Clostridium bifermentans
	Clostridium scindens		Escherichia coli	Clostridium symbiosum	Clostridium butyricum
	Clostridium sordelli		Fusobacterium ulcerans	Bacteroides vulgatus	Clostridium hathewayi
	Clostridium sporogenes		Pediococcus acidilactici	Bacteroides fragilis	Clostridium sporogenes
	Clostridium symbiosum		Proteus mirabilis	Acidamicoccus intestini	Clostridium symbiosum
	Clostridium tertium		Ruminococcus gnavus	Bacteroides ovatus	Clostridium tertium
	Enterococcus faecium			Proteus mirabilis	Clostridium scindens
	Enterococcus gallinarum			Streptococcus anginosus	Enterococcus avium
	Escherichia coli			Flavonifractor plautii	Enterococcus faecium
	Lactobacillus reuteri				Enterococcus faecalis
	Lactobacillus rhamnosus				Enterococcus gallinarum
	Lactobacillus zeae				Enterococcus thailandicus
	Ruminococcus gnavus				Escherichia coli
	Veillonella dispar				Flavonifractor plautii
	Proteus mirabilis				Lactobacillus reuterii
	Prevotella melanogenica				Morganella morgani
	Citrobacter freundii				Parabacteroides distasonis

Saggi di sensibilità antibiotici su isolati donatore

	CTX	CAZ	FEP	ETP	IPM	MEM	CS	TGC	AMP	TEC	VAN
C. freundii	<=1 S	<=1 S	<=1 S	<=0,5 S	<=0,25 S	<=0,25 S	<=0,5 S	<=0,5 S			
E. coli	<=1 S	<=1 S	<=1 S	<=0,5 S	<=0,25 S	<=0,25 S	<=0,5 S	<=0,5 S			
P. mirabilis	>=64 S	16 S	<=1 S	<=0,5 S		<=0,25 S	>=16 R	>=8 R			
E. faecium									<=2 S	<=0,5 S	<=0,5 S
E. gallinarum									<=2 S	<=0,5 S	4 R

	IPM	VAN	TZP	PIP	FOX	CLI	MRD	PEN	AMC
B. fragilis	0.06 S		128/4 R	128 R	8 S	64 R	2 S		
B. uniformis	2 S		16/4 S	16 S	64 R	1 S	32 R		
C. bifermentans	0.25 S	2 S	16/4 S			0.5 S	1 S	0.25 S	0,25/0,12 S
C. clostridioforme	1 S	2 S	16/4 S			0.5 S	0.5 S	0.25 S	0,5/0,25 S
C. scindens	0.5 S	2 S	16/4 S			0.5 S	0.5 S	0.25 S	1/0,5 S
C. sordellii	0.25 S	2 S	16/4 S			4 S	4 S	0.12 S	0,25/0,12 S
C. sporogenes	0.5 S	4 S	16/4 S			2 S	0.5 S	1 R	0,5/0,25 S
C. symbiosum	4 S	2 S	16/4 S			64 R	32 R	8 R	1/0,5 S
C. tertium	0.5 S	2 S	16/4 S			16 R	8	1 R	0,5/0,25 S

Pyrosequencing analysis donatore e ricevente - Phylum



Donatore:

Firmicutes 55 %

Bacteroidetes 40%

Proteobacteria 4%

Ricevente:

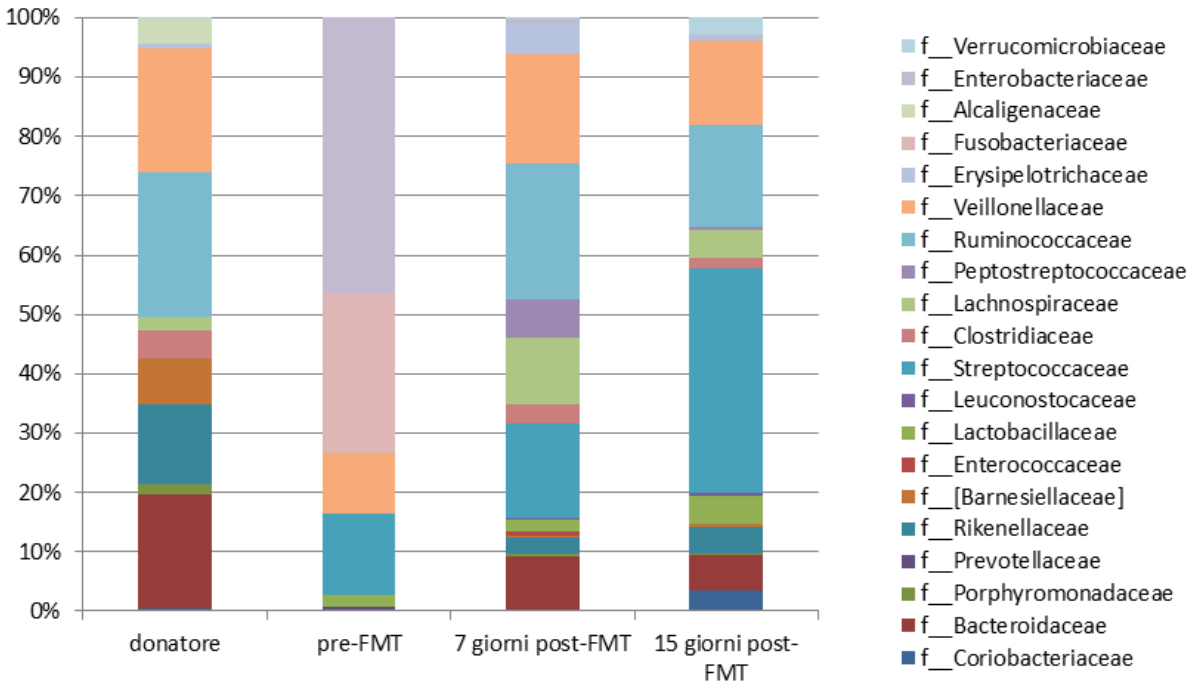
Proteobacteria 46%

Fusobacteria 27%

Firmicutes 26%

Bacteroidetes 11%

Pyrosequencing analysis donatore e ricevente - Famiglia



Donatore:
Ruminococcaceae (23%)
Veillonellaceae (20%)
Bacteoridaceae (19%)
Rikenellaceae (12%)
Clostridiaceae (5%)
Ricevente:
Enterobacteriaceae (46%)
Fusobacteriaceae (27%)
Streptococcaceae (13%)
Veillonellaceae (10%)

RICEVENTE a 7 GIORNI

Ruminococcaceae (19%), *Veillonellaceae* (16%), *Streptococcaceae* (13%), *Bacteroidaceae* (8%), *Lachnospiraceae* (9%), *Peptostreptococcaceae* (5%), *Clostridiaceae* (3%), *Lactobacillaceae* (2%) e *Rikenellaceae* (2%).

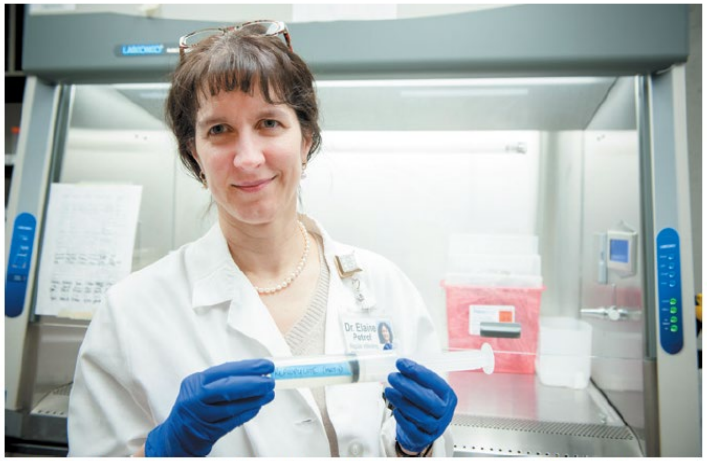
Petrof *et al. Microbiome* 2013, **1**:3
http://www.microbiomejournal.com/content/1/1/3

Microbiome

METHODOLOGY Open Access

Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: 'RePOOPulating' the gut

Elaine O Petrof[1*†], Gregory B Gloor[2†], Stephen J Vanner[1], Scott J Weese[3], David Carter[4], Michelle C Daigneault[5], Eric M Brown[5], Kathleen Schroeter[5] and Emma Allen-Vercoe[5]



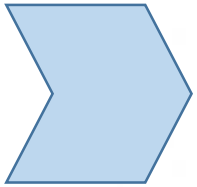
Elaine Petrof has invented a synthetic stool that could reset a patient's gut bacteria to cure infections.

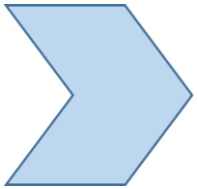
GASTROENTEROLOGY

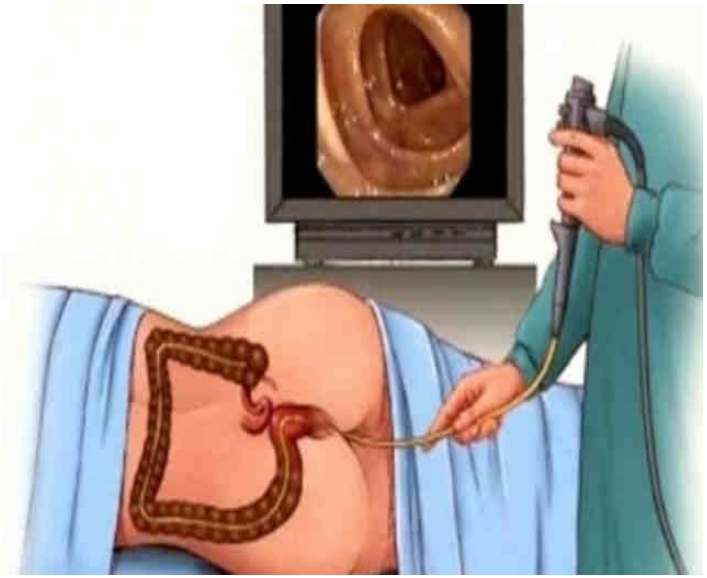
FDA gets to grips with faeces

Regulator triggers efforts to standardize faecal transplants.

Consorzio batterico







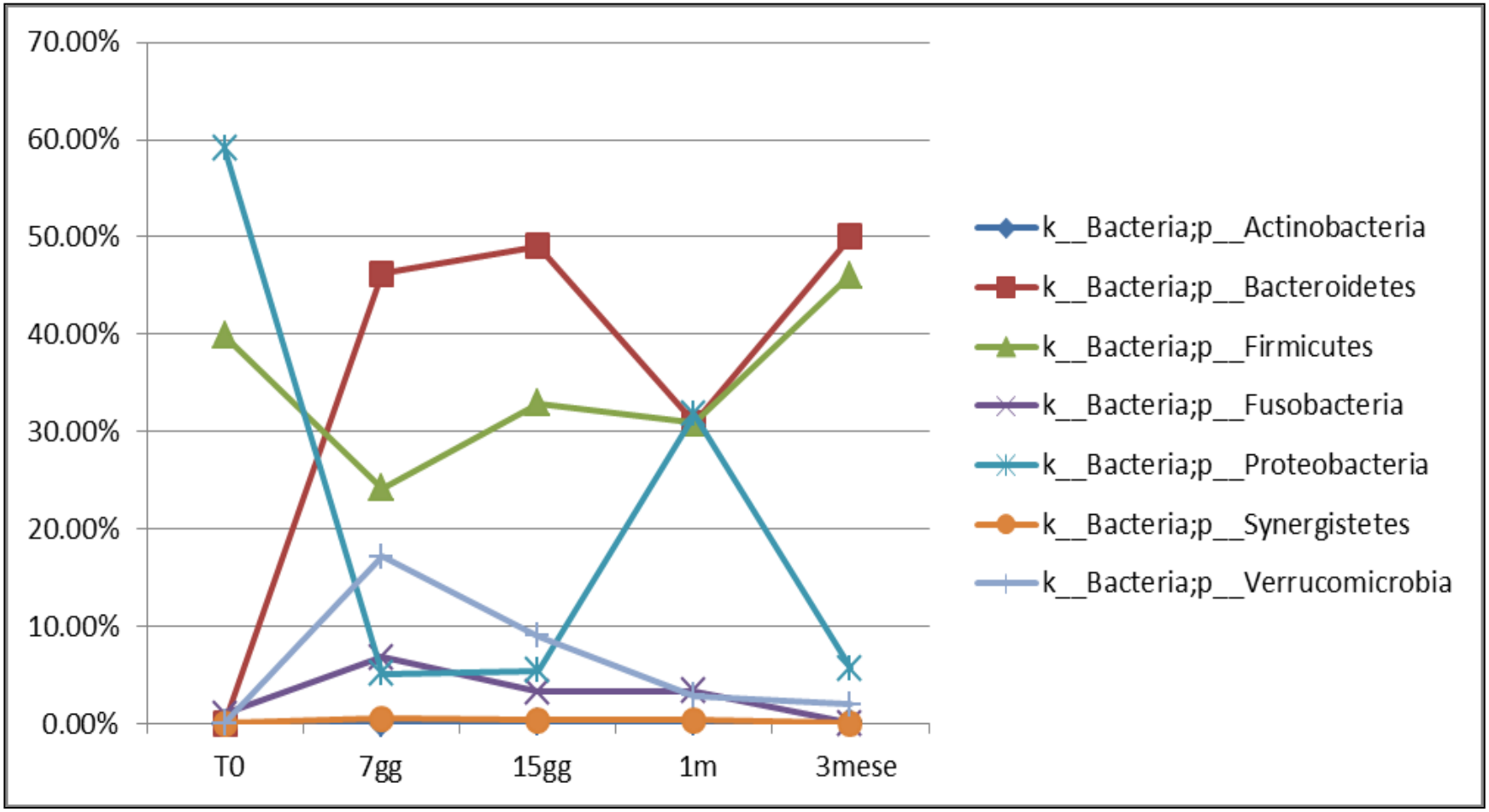
Colture batteriche
15 ceppi

Preparazione sospensione batterica
109 CFU/ml **Vf = 250 ml**

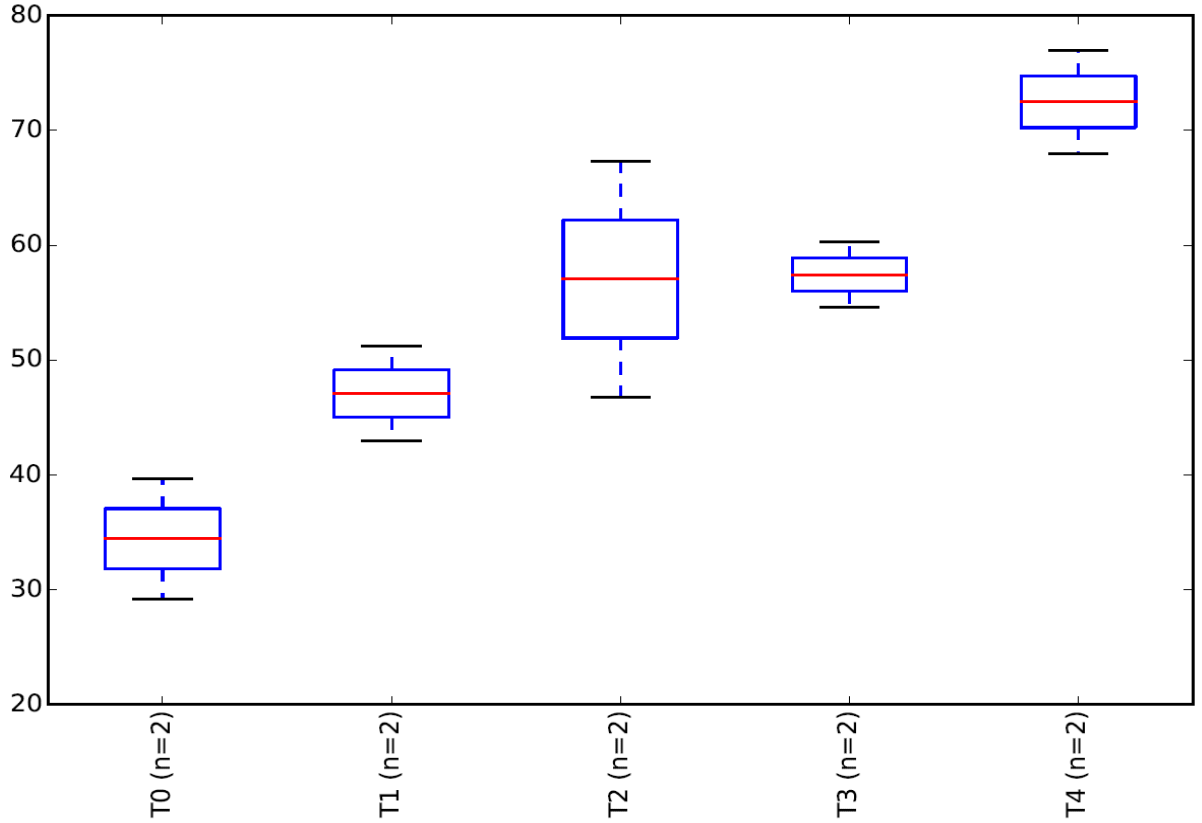
Composizione finale
- ***50% Firmicutes***
- ***30% Bacteroidetes***
- ***10% Proteobacteria***
- ***10% Actinobacteria***

Colonscopia

Consorzio batterico



Consorzio batterico



A tempo t0 il numero medio di specie rilevate è 35. Nei tempi di studio successivi si assiste ad un aumento in termini di diversità di specie passando dalle circa 50 descritte al T1 fino alle 70 rilevate al T4.

Considerazioni

- Studi mediante metagenomica hanno stimolato la “riscoperta” del microbiota umano intestinale attraverso la produzione di una grande quantità di dati.
- Metagenomica necessita di elevata conoscenza biostatistica ed un attento disegno dello studio
- La colturomica ha dimostrato in alcuni casi di superare la metagenomica.
- I dati sono talvolta discordanti
- La colturomica è lunga e laboriosa, ma è possibile controllare i ceppi isolati.

Considerazioni

LAB FMT AND CULTUROMICS Team

- Dott. Gianluca Quaranta
- Dott. Giovanni Fancello

Gemelli

Fondazione Policlinico Universitario Agostino Gemelli IRCCS
Università Cattolica del Sacro Cuore

Vincenzo Tiberio

“Lunga e difficile è la via della ricerca, ma alla base di tutto c’è l’amore”