



Dipartimento  
**Medicina Veterinaria**  
Produzioni Animali

# Antibiotico

# Resistenza



**PROF : IOVANE GIUSEPPE**, ordinario di malattie infettive  
Direttore scuola specializzazione  
Membro CSS  
Membro CNSSV



# Antibiotico resistenza: ieri

NO. 3713, DEC. 28, 1940

NATURE

837

## LETTERS TO THE EDITORS

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IN THE PRESENT CIRCUMSTANCES, PROOFS OF "LETTERS" WILL NOT BE SUBMITTED TO CORRESPONDENTS OUTSIDE GREAT BRITAIN.

### An Enzyme from Bacteria able to Destroy Penicillin

*B. coli*, it was not necessary to crush the organism in the bacterial mill in order to obtain the enzyme from it; the latter appeared in the culture fluid.

E. P. ABRAHAM.  
E. CHAIN.

*"There is probably no chemotherapeutic drug to which in suitable circumstances the bacteria cannot react by in some way acquiring 'fastness' [resistance]."*

—Alexander Fleming, 1946

# Sampling the Antibiotic Resistome

Vanessa M. D'Costa,<sup>1</sup> Katherine M. McGrann,<sup>1</sup> Donald W. Hughes,<sup>2</sup> Gerard D. Wright<sup>1\*</sup>

*Science* **311**, 374 (2006);

DOI: 10.1126/science.1120800

L'antibiot  
resistenz  
antica

LETTER TO NATURE

doi:10.1038

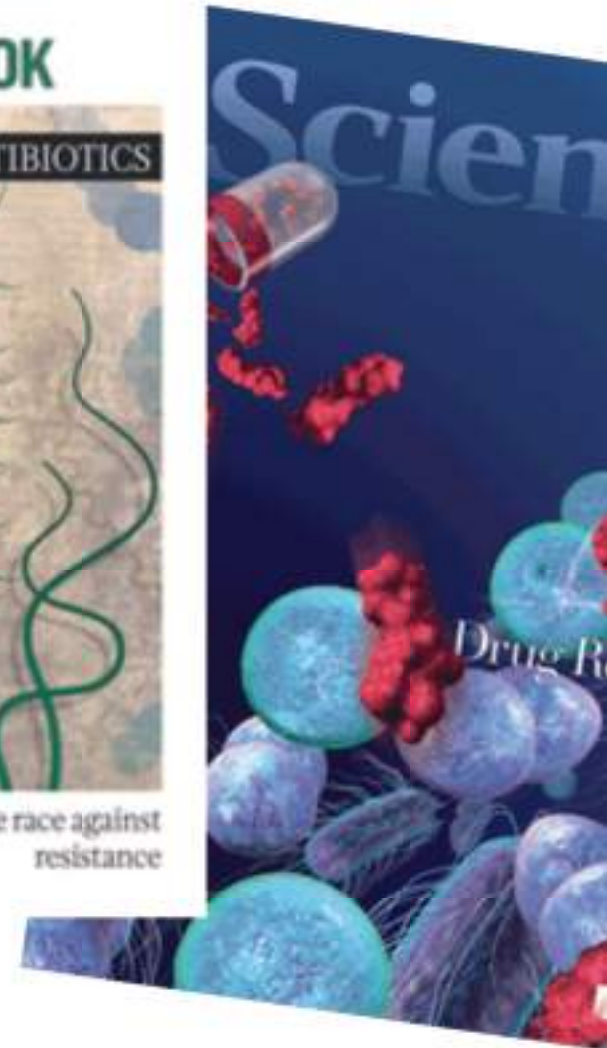
## Antibiotic resistance is ancient

Vanessa M. D'Costa<sup>1,2\*</sup>, Christine E. King<sup>3,4\*</sup>, Lindsay Kalan<sup>1,2</sup>, Mariya Morar<sup>1,2</sup>, Wilson W. L. Sung<sup>4</sup>, Carsten Schwabe<sup>5</sup>, Duane Froese<sup>5</sup>, Grant Zazula<sup>6</sup>, Fabrice Calmels<sup>5</sup>, Regis Debruyne<sup>7</sup>, G. Brian Golding<sup>4</sup>, Hendrik N. Pöhlmann<sup>4</sup>

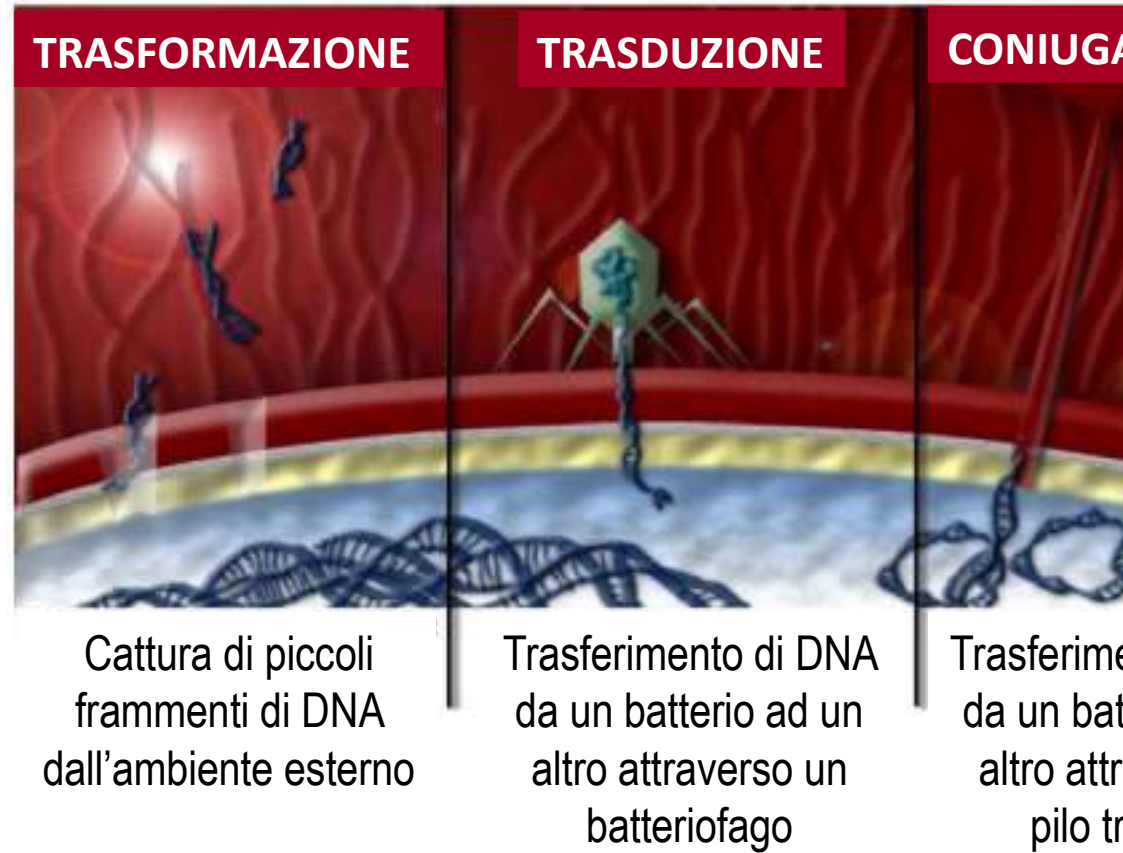
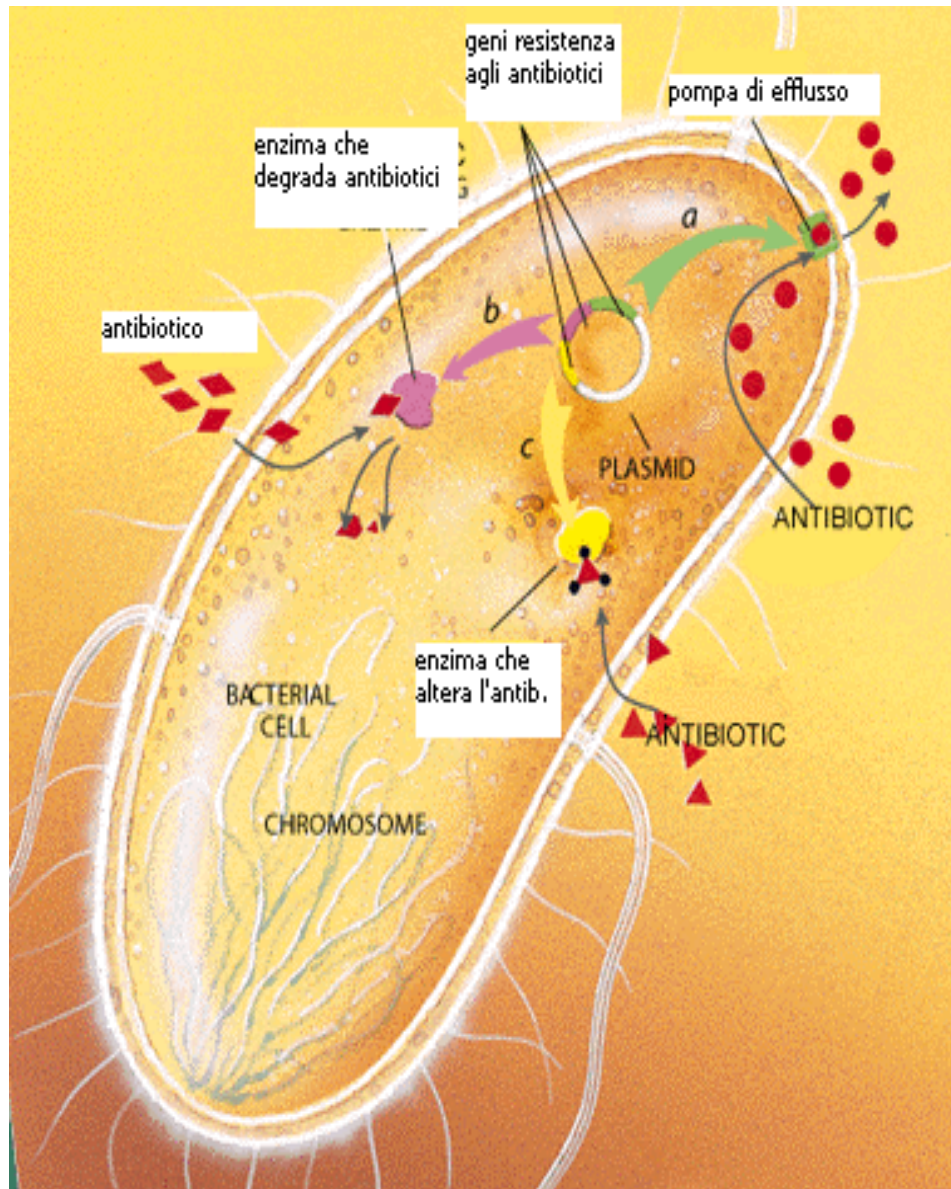


UNIVE  
DEGLI  
FEDER

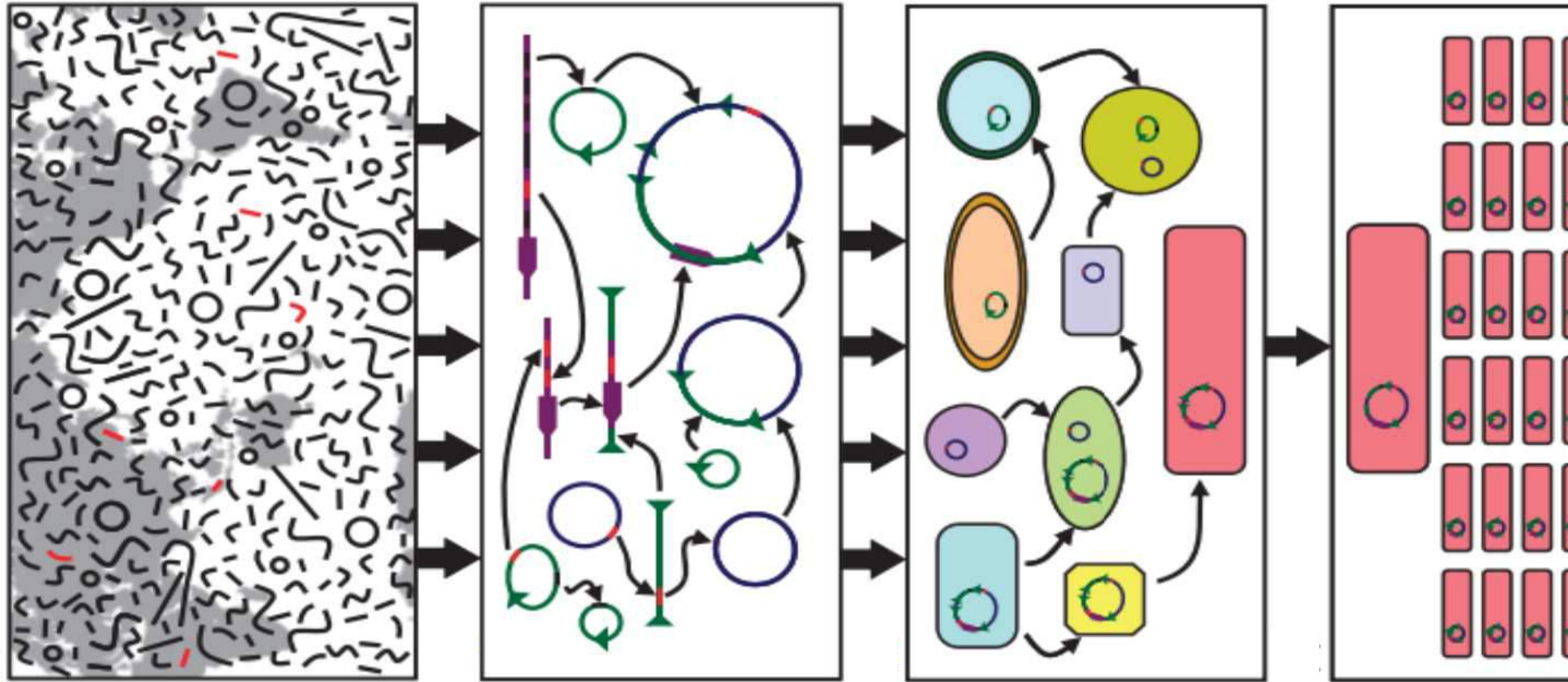
# Antibiotico resistenza: oggi



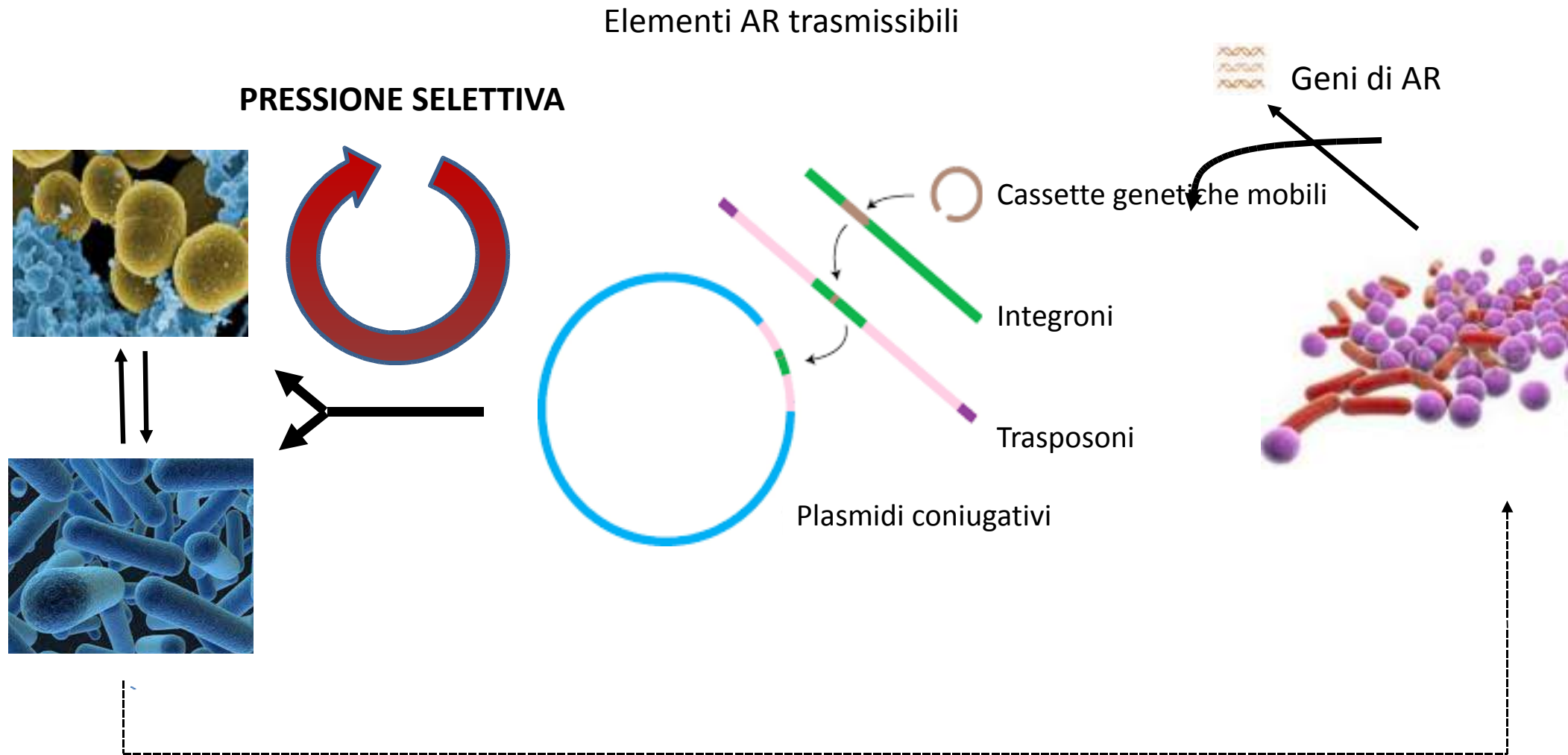
# Basi genetiche e molecolari dell'antibiotico resist



# Il resistoma

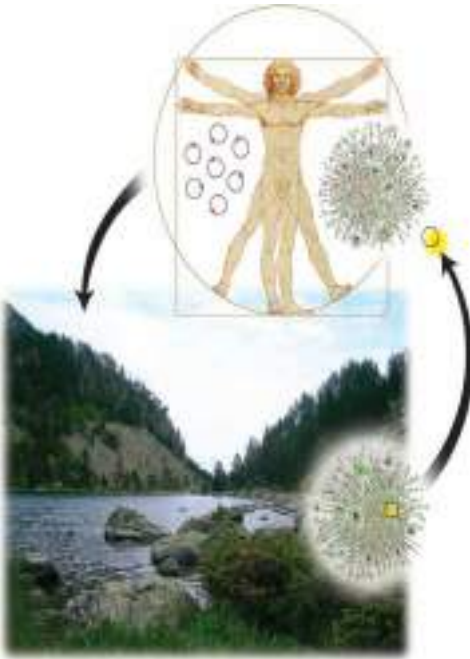


# Meccanismi e origini dell'antibiotico resistenza



Adattato da Davies e Davies. *Micr*  
Mol Biol Rev. 2010 doi: 10.1128/M

# Dispersione degli antibiotici nell'ambiente



Antibiotico	Persistenza/mobilità	Fanghi	Fiumi	Acqua del suolo	Acqua potabile	Pesci	Suolo	Colture	Esempi di antibiotici
Cloranfenicolo	-/+	nd	✓	X	nd	nd	nd	nd	Cloranfenicolo
Fluoro-chinoloni	+/-	✓	✓	x	x	nd	✓	nd	Ciprofloxacina
β-lattamici	+/-	nd	x	X	X	nd	nd	nd	Amoxicillina, penicilline
Macrolidi	+/+	✓	✓	X	nd	nd	nd	nd	Azitromicina, claritromicina
Sulfamidici	+/+	✓	✓	✓	X	nd	✓	✓	Sulfometazolo
Teracicline	+/-	nd	✓	x	X	✓	✓	✓	Tetracicline

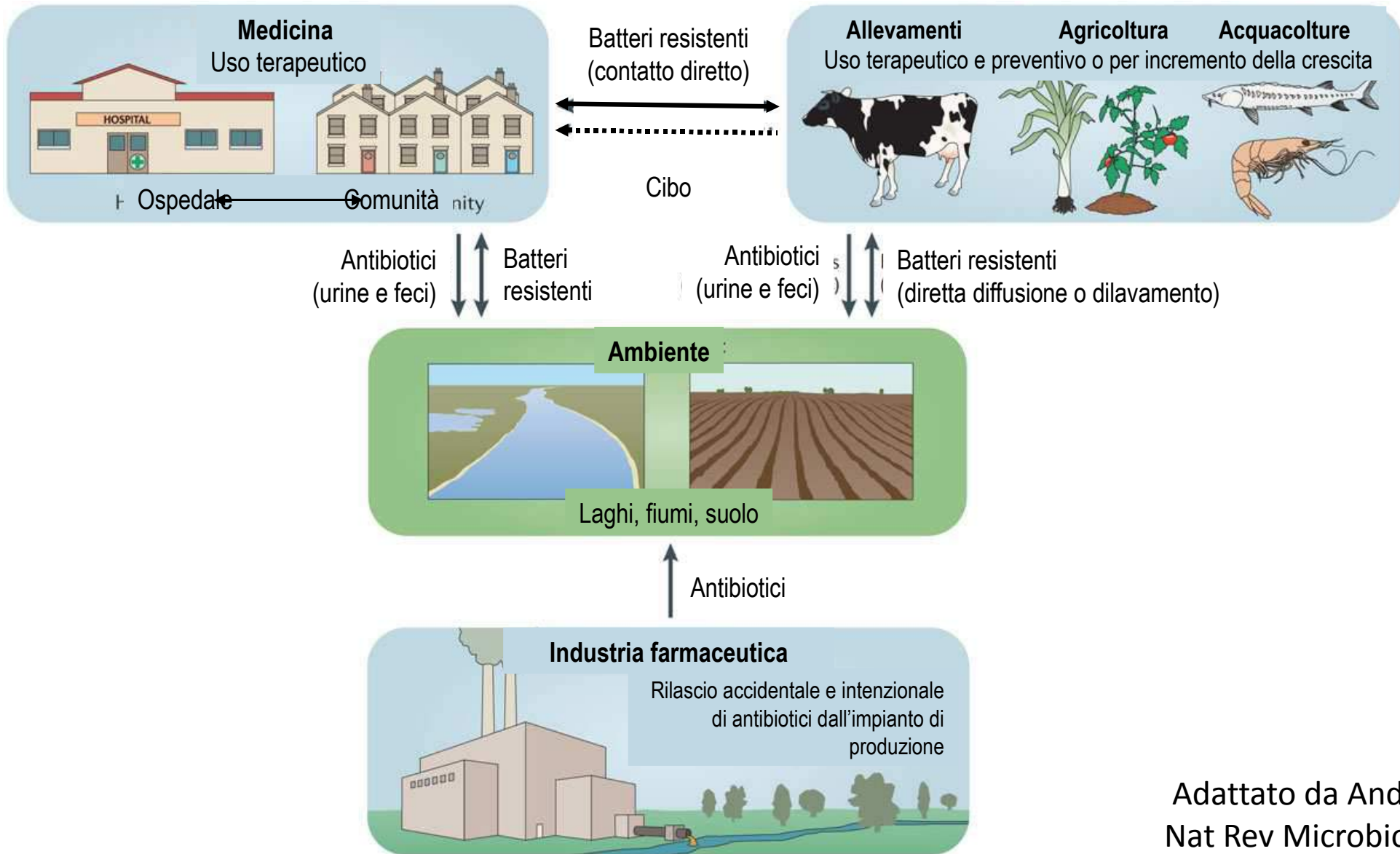
nd, non determinato; ✓, rilevato, x, non rilevato

Martínez JL.  
Science. 2008 doi:  
10.1126/science.1159483.

Adattato da Wellington et al., Lancet  
Dis. 2013 doi: 10.1016/S1473-3099(12)70317-1.

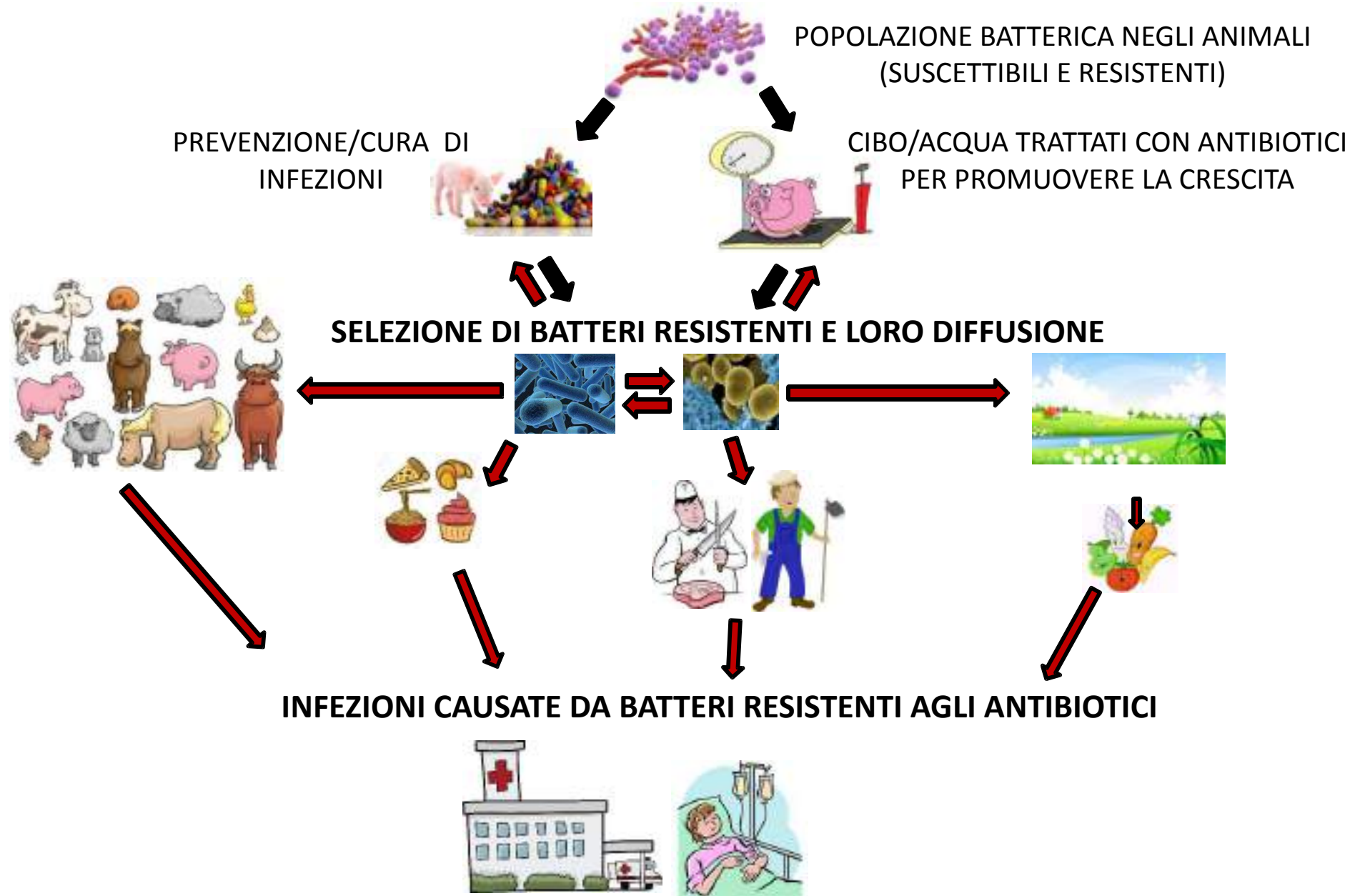


# Antibiotico resistenza: dall'ambiente alla clinica

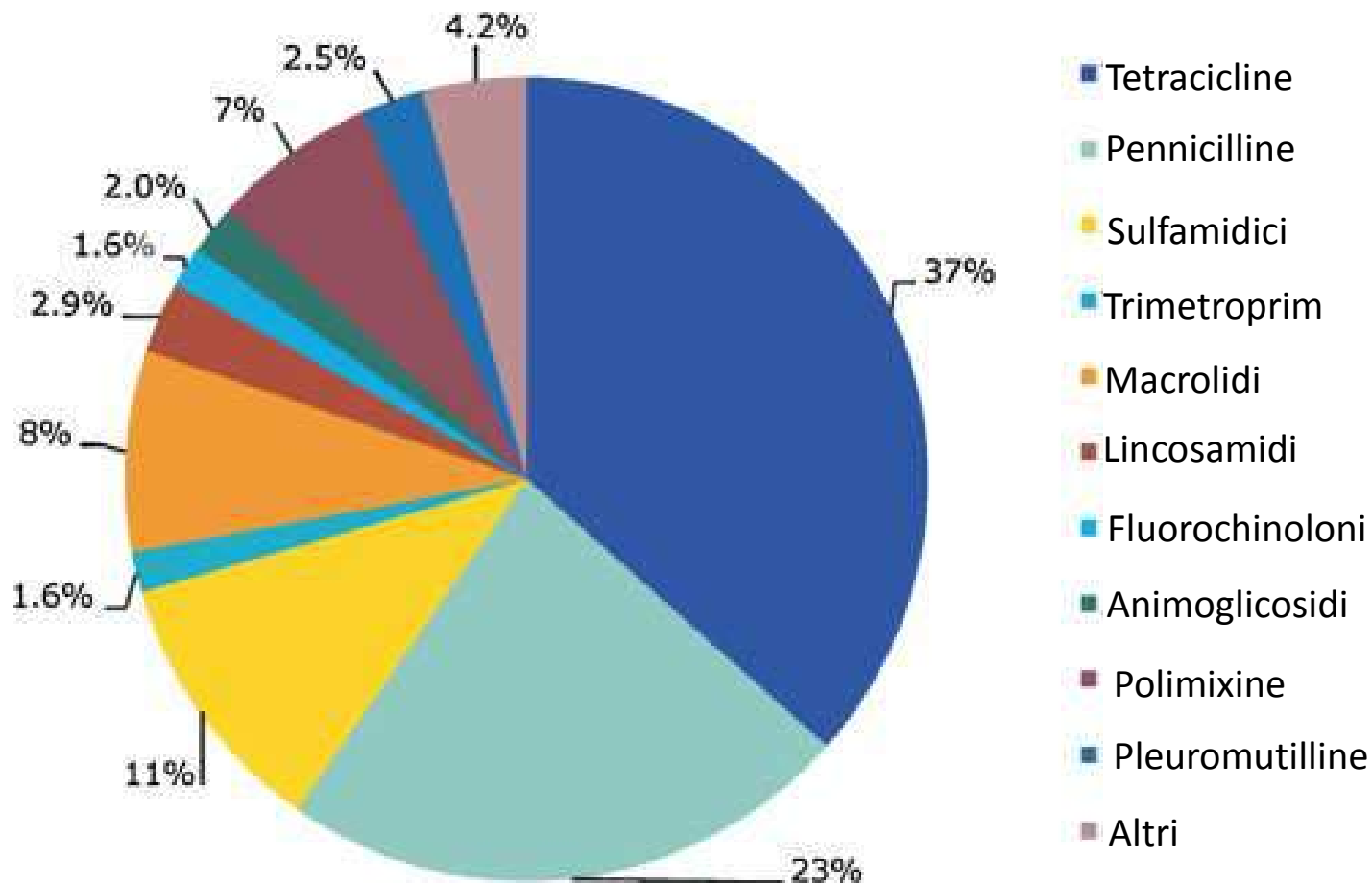


Adattato da Ander  
Nat Rev Microbiol.  
10.1038/nrmicro32

# Batteri resistenti agli antibiotici: dagli animali all'uomo



# Vendita di antibiotici negli allevamenti ( 1200 ton



## Principali batteri multiresistenti di origine animale/alimentare

Salmonelle non tifoidi e *Campylobacter jejuni* resistenti ai fluorochinoloni

*Clostridium difficile*

Enterobatteriaceae Resistenti ai  $\beta$ -lattamici (E)

*Staphylococcus aureus* Resistente alla meticillina

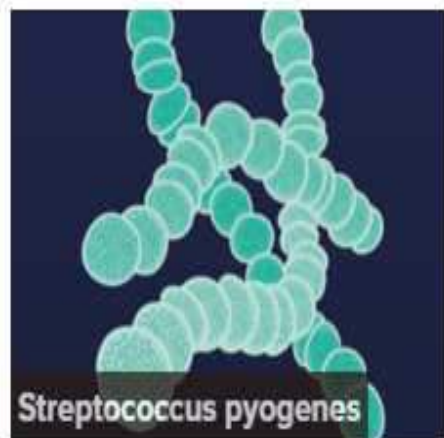
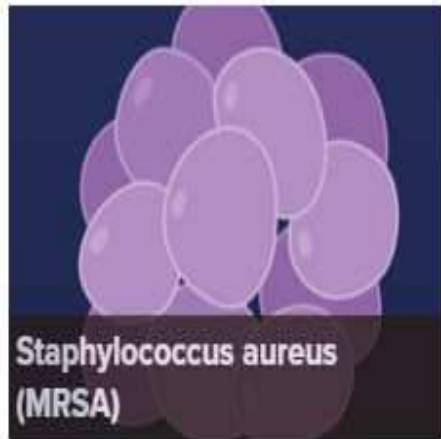
Catry et al., 2015. Int J Antimicrob Agents. doi: 10.1016/j.ijantimicag.2015.06.005.

# Antibiotico resistenza: una minaccia globale

MDR, Multi Drug Resistant (microrganismi resistenti ad almeno uno degli antibiotici di tre o più famiglie diverse)

XDR, Extensively Drug-Resistant (microrganismi sensibili solamente ad una o due classi di antibiotici)

PDR, Pan Drug-Resistant (microrganismi resistenti a tutte le classi di antibiotici)



50.000 morti anno -eu 1,5 miliardi euro

usa-20 miliardi \$

# Antibiotico resistenza: i patogeni più critici

**E** *Enterococcus faecium* (resistenti alla Vancomicina, VRE)

**S** *Staphylococcus aureus* (resistenti alla Meticillina/Vancomicina, MRSA/VRSA)

**K/C** *Klebsiella pneumoniae/Clostridium difficile* (resistenti ai Carbapenemici)

**A** *Acinetobacter baumannii*

**P** *Pseudomonas aeruginosa*

**E** *Enterobacter* spp.



I patogeni ESKAPE sfuggono (*escape*) all'effetto degli agenti antimicrobici perché resistenti

Boucher HW, et al. *Clin Infect Dis* 2009;48:1-12. "ESKAPE"

Peterson LR. *Clin Infect Dis* 2009;49:992-3. "ESCAPE"

# Antibiotico resistenza: sorveglianza 2014

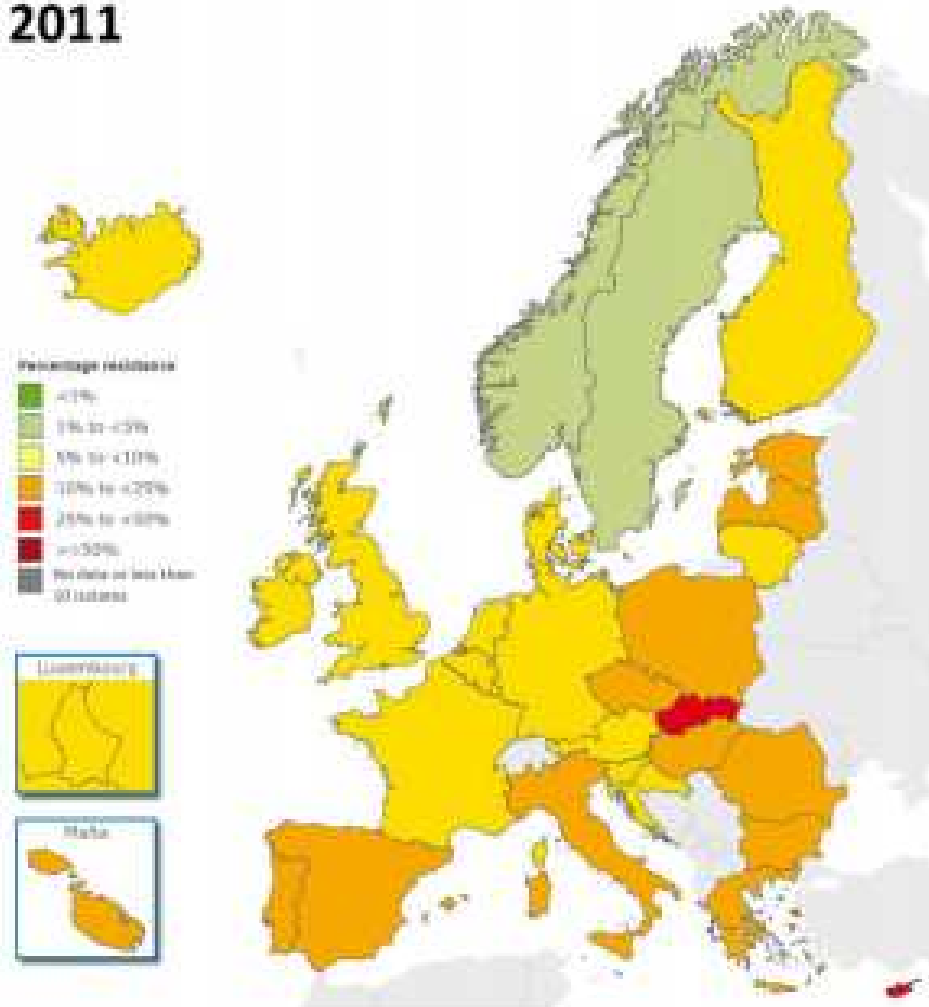


# Antibiotico resistenza: *Escherichia coli*

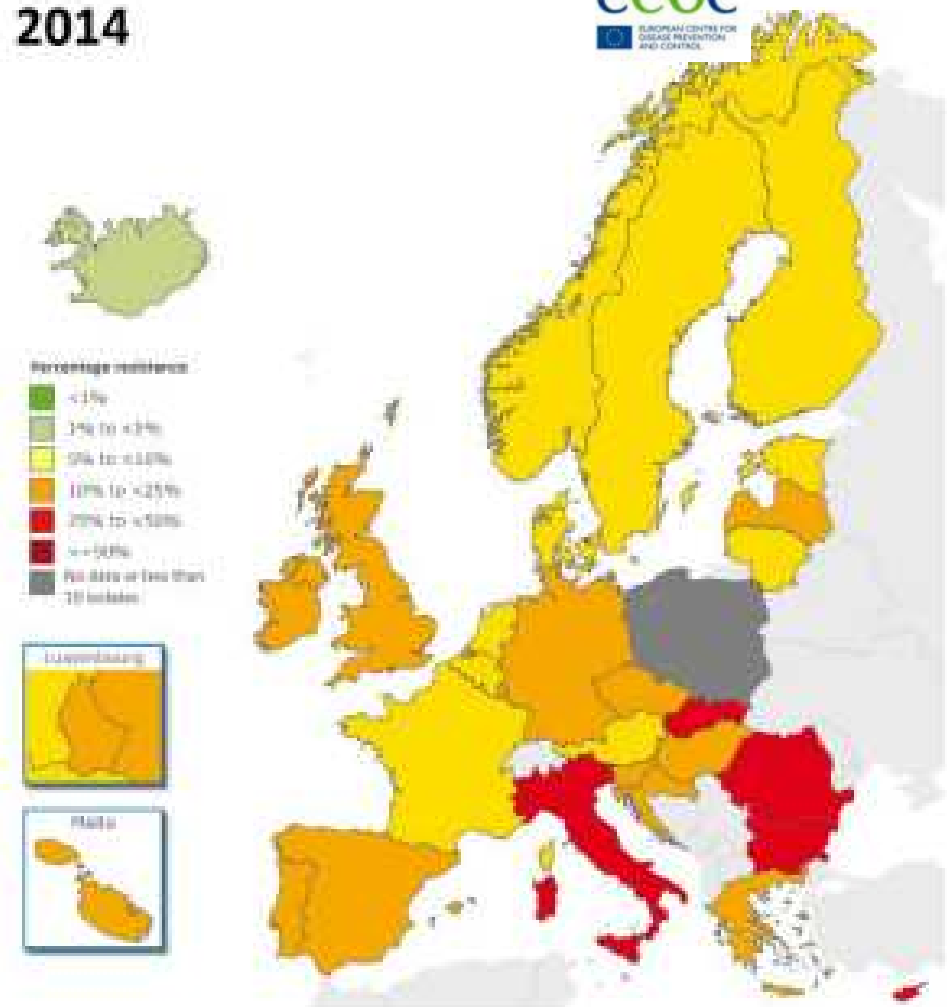
Percentuale di isolati resistenti alle cefalosporine di terza generazione



2011



2014



# Antibiotico resistenza: *Klebsiella pneumoniae*

Percentuale di isolati resistenti ai carbapenemi



2011



2014





# Antibiotico resistenza: *Staphylococcus aureus*

Percentuale di isolati resistenti alla meticillina



2011



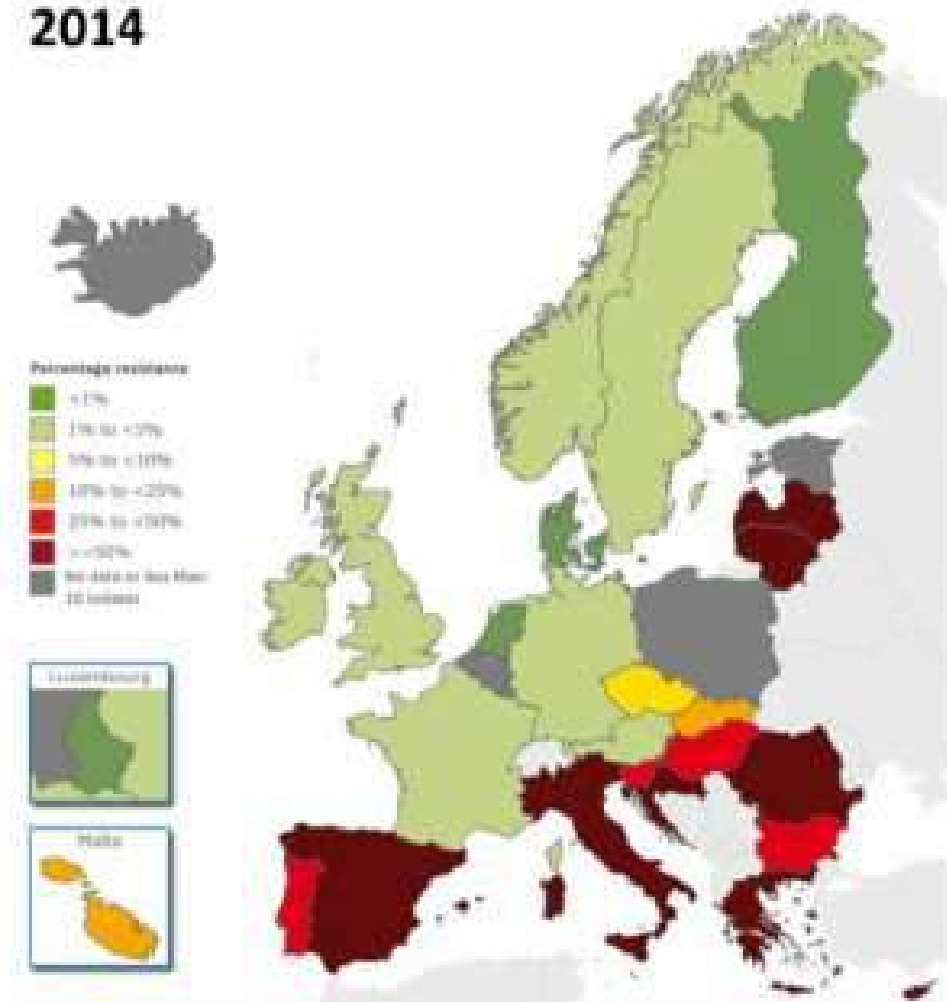
2014



# Antibiotico resistenza: *Acinetobacter* spp.

Percentuale di isolati resistenti ai fluorochinoloni, aminoglicosidi e carbapenemi

2014



# Antibiotico resistenza: che fare?



**2001:** congresso WHO a Ginevra. STRATEGIE PER LA LOTTA ALL'ANTIBIOTICO RESISTENZA



**2005:** WHO SOLLECITA UN'AZIONE SULLA ANTIBIOTICO RESISTENZA



**2008:** il gruppo di lavoro ECDC ed EMEA stilano il RAPPORTO «The bacterial challenge: tir



**2011:** World Health Day



**2013:** DECISIONE N. 1082/2013/UE DEL PARLAMENTO EUROPEO E DEL CONSIGLIO



**2015:** G7 DEI MINISTRI DELLA SANITÀ sulla resistenza agli antimicrobici

# Antibiotico resistenza: sensibilizzazione nel

Qualunque utilizzo di agenti antimicrobici ha le potenzialità di selezionare organismi

**Antibiotici.**  
Usali in modo corretto,  
mai per curare  
raffreddore e influenza.

**GIORNATA  
EUROPEA  
DEGLI ANTIBIOTICI**



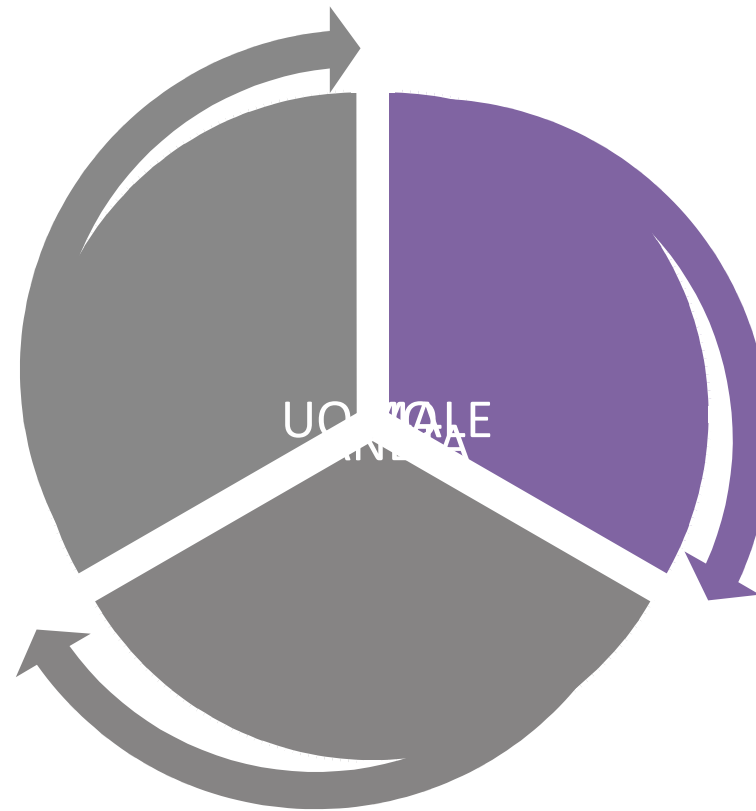
 UN'INIZIATIVA EUROPEA  
PER LA SALUTE



10 febbraio 2016

# One health approach

"Lo sforzo di collaborazione tra discipline diverse - che lavorino a livello locale, nazionale e globale - al fine di raggiungere un livello di salute ottimale per le persone, gli animali e l'ambiente"



# Approccio One-Health

## Un approccio globale ad un problema mondiale

World Health Organization



Il piano di azione deve essere attuato a livello nazionale ed internazionale.

Deve coinvolgere tutti i settori rilevanti: salute dell'uomo, animali, agricoltura, ambiente e ricerca.

Il World Health Assembly ha individuato cinque obiettivi:

- migliorare la consapevolezza e la comprensione della resistenza antimicrobica attraverso una **comunicazione**, l'educazione e la formazione
- rafforzare le conoscenze attraverso la sorveglianza ed incrementare la ricerca
- ridurre l'incidenza di infezioni attraverso efficaci misure igienico-sanitarie e prevenzione delle infezioni
- ottimizzare l'uso di farmaci antimicrobici nella salute umana e animale
- incrementare gli investimenti per lo sviluppo di nuovi farmaci, strumenti diagnostici, vaccini e altri interventi

# Approccio One-Health

Un approccio globale ad un problema mondiale



**UNA ALLEANZA TRIPART**

[http://apps.who.int/iris/bitstream/10665/193736/1/9789241509763\\_eng.pdf?ua=1&ua=1](http://apps.who.int/iris/bitstream/10665/193736/1/9789241509763_eng.pdf?ua=1&ua=1)

# Antibiotico resistenza

## Un approccio globale ad un problema mondiale



Prevenzione delle infezioni microbiche e della loro propagazione

Utilizzo prudente degli antibiotici, favorendo le diagnosi microbiologiche

Queste indicazioni si intendono estese all'industria della salute animale, veterinari e allevatori



Produzione di antimicrobici di alta qualità in ambito medico e veterinario

Rafforzare la ricerca (sviluppo di nuovi antimicrobici, vaccini, trattamenti antimicrobici alternativi)

Sistema di sorveglianza a livello nazionale ed internazionale, particolarmente sui patogeni di difficile trattamento terapeutico

Sviluppare un network per il rapido scambio di ricerche e dati



Protocolli standard e linee guida per evidenziare la presenza di agenti antimicrobici e residui nell'ambiente (acqua, mangimi per animali terrestri ed acquatici)



# One health approach

Tutti dobbiamo cambiare le nostre abitudini sugli antibiotici come



Pazienti



Dottori/ricercatori



Veterinari/padroni di animali domestici



Genitori



Divulgatori di informazione



**«Non dimentichi di prendere, uscendo, una manciata  
dei nostri antibiotici in omaggio.»**

Infezioni resistenti agli antibiotici e  
approccio one health:  
una strategia senza confini per  
combattere i superbatteri

G A P = global action plan

BIOFABEN-Biosicurezza e benessere

Riduzione del 29% settore cunicolo

Dosi antibiotici usati un anno in Italia  
6.670 485



Importante il ruolo del  
laboratorio di batteriologia  
nella pratica clinica veterinaria





## Elenco Prestazioni Batteriologia

### DESCRIZIONE

- Esame colturale per la ricerca di batteri aerobi
- Esame colturale per la ricerca di batteri anaerobi
- Esame colturale per la ricerca di batteri aerobi completo di antibiogramma
- Esame colturale per la ricerca di batteri anaerobi completo di antibiogramma
- Emocolture
- Esame coltura sierologica di anticorpi gassose
- Diploidi uretrali per lo screening della infezione urinaria
- Diploidi uretrali per lo screening delle infezioni urinarie completo di antibiogramma
- Esame coltura per micobatteri



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## Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)

Original article

<http://dx.doi.org/10.1016/j.apjtb.2015.11.012>

## An update on microbiological causes of canine otitis externa in Campania Region, Italy

Luisa De Martino<sup>1\*</sup>, Francesca Paola Nocera<sup>1</sup>, Karina Mallardo<sup>1</sup>, Sandra Nizza<sup>1</sup>, Eleonora Masturzo<sup>1</sup>, Filomena Fiorito<sup>1</sup>, Giuseppe Iovane<sup>1</sup>, Piergiorgio Catalanotti<sup>2</sup>



CrossMark

**Results:** Thirty-one out of 122 dogs were positive for yeast species (25.4%, 95% confidence interval (CI): 18.2%–34.2%) with a higher prevalence of *Malassezia pachydermatis* (21/31 isolates, 67.7%, CI: 48.5%–82.7%), and a total of 91 out of 122 dogs were positive for bacterial species (74.6%; CI: 65.8%–81.8%) with a higher prevalence of *Staphylococcus pseudintermedius* (45/143 isolates, 31.5%, CI: 24.1%–39.8%). These

Number and percentage of isolated yeasts from dogs with otitis externa.

Yeasts	No. of isolates	Percent (%)	95% CI
<i>M. pachydermatis</i>	21	67.74	48.54–82.68
<i>Candida parapsilosis</i>	4	12.90	4.22–30.76
<i>Candida tropicalis</i>	2	6.45	1.13–22.84
<i>Candida glabrata</i>	1	3.23	0.17–18.51
<i>Candida albicans</i>	1	3.23	0.17–18.51
<i>Aspergillus niger</i>	1	3.23	0.17–18.51
<i>Exophiala</i> spp	1	3.23	0.17–18.51
Total	31		



*Ippologia, Anno 21, n. 3, Settembre 2010*



## **Presenza di stafilococchi meticillina-resistenti in cavalli clinicamente sani**

*Karina Mallardo, Sandra Nizza, Bruna Facello, Emilia Pierni, Eleonora Masturzo,  
Ugo Pagnini, Giuseppe Iovane, Luisa De Martino*

*1 Dipartimento Patologia e Sanità Animale, Sezione di Malattie Infettive, Facoltà di Medicina Veterinaria, Università di Napoli "Federico II"*

*2 Dipartimento di Struttura, Funzioni e Biotecnologie*

*3 Medico Veterinario, Libero professionista, Avellino*

*4 Istituto Zooprofilattico Sperimentale del Mezzogiorno-Portici, Napoli*

### **RIASSUNTO**

La meticillina è stata introdotta per la prima volta in medicina umana negli anni '50 per il trattamento di infezioni causate da *Staphylococcus aureus* penicillinasi-resistente. La resistenza alla meticillina è mediata da una proteina, la Penicillin binding protein (PBP 2a) che ha una bassa affinità per gli antibiotici beta-lattamici. La proteina PBP 2a è codificata dal gene *mecA*.

Lo scopo del nostro studio è stato quello di documentare la presenza di ceppi di stafilococchi meticillino-resistenti (MRS) isolati da tamponi nasali equini e del personale addetto al governo degli animali e di determinare la relazione genetica tra questi. Dai tamponi nasali equini sono stati isolati complessivamente 42 ceppi di stafilococchi meticillino-resistenti, di cui *S. xylosus* (47,6%), *S. sciuri* (40,5%), *S. lentus* (10,2%), *S. aureus* (2,4%) e *S. capitis* (2,4%). Tutti gli isolati contenevano il gene *mecA* come confermato dall'analisi di PCR.

**Il nostro studio è un'ulteriore conferma che i cavalli possono fungere da reservoir di MRS e da fonte di infezioni e reinfezioni per**





J Vet Diagn Invest 22:77–82 (2010)

## **Methicillin-resistant staphylococci isolated from healthy horses and horse personnel**

Luisa De Martino, Maria Lucido, Karina Mallardo, Bruna Facello, Michelina Mallardo,  
Giuseppe Iovane, Ugo Pagnini, Maria Antonietta Tufano,<sup>1</sup> Piergiorgio Catalanot

**Abstract.** Methicillin-resistant staphylococci (MRS) were isolated from nasal swabs of 56 of 159 (35.2% confidence interval [CI]: 27.9–43.2%) healthy horses. Two nasal swabs were collected from each horse; 43 (27%; 95% CI: 20.5–34.8%) of the cohort were colonized by MRS strains in 1 nostril, while in the remaining 159 (8.2%; 95% CI: 4.6–13.9%), different or identical MRS strains were isolated in both nostrils. Of 1 humans in close contact with the horses tested, 4 (13.8%; 95% CI: 4.5–32.6%) were found to be carriers of MRS. All isolates were coagulase negative with the exception of 2 coagulase-positive MRS strains, *Staphylococcus aureus* and *Staphylococcus pseudintermedius*, both isolated from horses. To assay the methicillin resistance susceptibility test to oxacillin with standardized disk diffusion method, a PBP-2a latex agglutination test, methicillin resistance gene (*mecA*) polymerase chain reaction assay were performed. Pulsed-field electrophoresis patterns of isolates from horses and humans in close contact with the horses revealed similarities. The results suggest evidence of transmission between animals, from animals to humans, and vice versa.

**PAPER**
**Antibiotic susceptibility of haemolytic *E. coli* strains isolated from diarrhoeic faeces of buffalo calves**

Sandra Nizza, Karina Mallardo, Annarossaria Marullo, Valentina Iovane, Luisa De Martino, Ugo Pagnini  
 Dipartimento di Patologia e Sanità Animale, Università di Napoli Federico II, Italy

**Abstract**

We investigated the antibiotic resistance of a collection of 94 strains (55.8%) of haemolytic *Escherichia coli* (*E. coli*) isolated in 1659 diarrhoeic faecal samples from buffalo (*Bubalus bubalis*) calves. Bacterial colonies on McConkey and EHEC agar that showed the morphology of *E. coli* were biochemically tested and then, furtherly classified as haemolytic, using PCR-based assays for enterohemorrhagic *E. coli* hly (*hly*) (*hly*) virulence gene. When the pathogenic isolates were tested for their susceptibility to 13 different antibiotics, each tested isolate was found to be highly resistant to more than three antibiotics. In fact, absolute resistance (100% of resistance) to penicillin G, lincomycin, neomycin, was detected. Amoxicillin/clavulonic acid and ampicillin were found to be moderately effective against the majority of isolates (46.8% of resistance). Thirty-two (34%) of the haemolytic *E. coli* strains were phenotypically resistant to tetracycline. None of the isolated strains of *E. coli* was resistant to colistin sulfate. We conclude that the high prevalence of antimicrobial resistance detected in our study is a source of concern, and cautious use of antibiotics in food producing animals is highly recommended.

**Table 1. Serogroups of *Escherichia coli* isolated from diarrhoeic faecal samples from buffalo (*Bubalus bubalis*) calves.**

Serotypes of EHEC	Positive strains	%	95% Confidence interval
O157	40	42.5	32.5-53.2
O26	4	4.3	1.4-11.1
O111	4	4.3	1.4-11.1
O128	12	12.8	7.1-21.6
O103	0	0.0	0.1-4.9
ND	34	36.2	26.7-46.8

ND: not detected.

**Table 2. Resistance to thirteen antimicrobial agents among 94 haemolytic isolates of *E. coli*.**

Antibiotics	No. of resistant isolates	%	95% Confidence interval
Nalidixic acid (NA)	22	23.4	15.6-33.5
Anoxicillin/clavulonic acid (AMC)	44	46.8	36.5-57.3
Ampicillin (AMP)	44	46.8	36.5-57.3
Apramycin (APR)	20	21.3	13.8-31.2
Colistin sulfate (CS)	0	0	0.1-4.9
Enrofloxacin (ENR)	18	19.1	12.0-28.8
Gentamicin (GN)	8	8.5	4.0-16.6
Tetracycline (TE)	32	34.0	24.8-44.6
Trimethoprim/Sulphamethoxazole (SXT)	14	14.9	8.7-24.1
Ceftiofur (EPT)	4	4.3	1.4-11.1
Neomycin (N)	94	100	95.1-99.9
Lincomycin (MY)	94	100	95.1-99.9
Penicillin G (P)	94	100	95.1-99.9





Journal of Agricultural Science and Technology A, 1 (2011) 997-1003

Earlier title: Journal of Agricultural Science and Technology, ISSN 1039-1250



## Lethal Co-infection of Rotavirus and *E. coli* O157:H7 in Mediterranean Buffalo Calves

L. De Martino, F. Florito, G. Pionelli, S. Nizza, K. Mallardo, R. Schietini, S. Montingano, G. Iovine and U. Pagani

Department of Pathology and Animal Health, Section of Infectious Diseases, School of Veterinary Medicine, University of Naples "Federico II", Naples 80137, Italy

Received: April 6, 2011 / Published: November 20, 2011.

**Abstract:** In this paper, we reported lethal episodes in buffalo calves, due to rotavirus and concurrent infection with *E. coli* O157:H7, in a closed community in Italy. A first detection of group A rotavirus infection was made by using an immunochromatographic assay, the virus isolation was performed on Monkey kidney cells (MA-104), and the rotavirus isolates were G and P characterized by a reverse transcription (RT)-PCR assay using a nested amplicles method. Co-infection with *E. coli* strains was demonstrated by classical bacteriological procedures. The *E. coli* isolates were analysed by Vero cell assay and PCR. This study showed the presence of group A rotavirus of G6-P5 serotypes and verotoxin-producing or non-producing *E. coli* O157:H7 in faecal samples. So far the concomitant presence of rotavirus and *E. coli* O157:H7 in cases of enteritis in *Bubalus bubalis* in Italy is not reported in the literature, as well as the mortality of buffalo calves probably due to an additional virulence factor, Shiga toxin 1 (stx1) gene, of the *E. coli* O157:H7 strains.

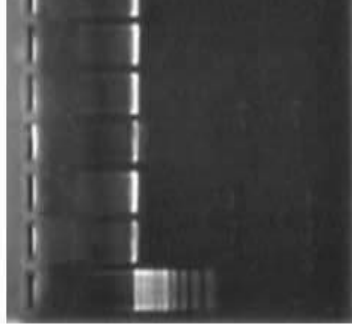
## Lethal Co-infection of Rotavirus and *E. coli* O157:H7 in Mediterranean Buffalo Calves

L. De Martino, F. Fiorito, G. Pisanelli, S. Nizza, K. Mallardo, R. Schettini, S. Montanaro, G. Iovane and U. Paglianti

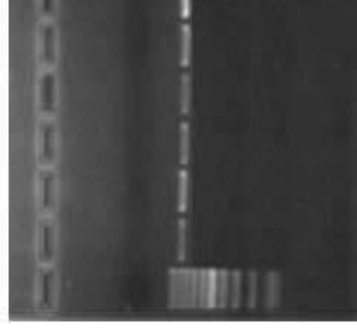
**Table 1** Results of the PCR of the isolated *E. coli* O157:H7.

Specimen	<i>stx1</i>	<i>stx2</i>	EHEC O157:H7 ( <i>hly<sub>E</sub>A</i> )
1	-	-	+
2	-	-	+
3	+	-	+
4	+	-	+
5	-	-	+
6	+	-	+
7	-	-	+
8	+	-	+

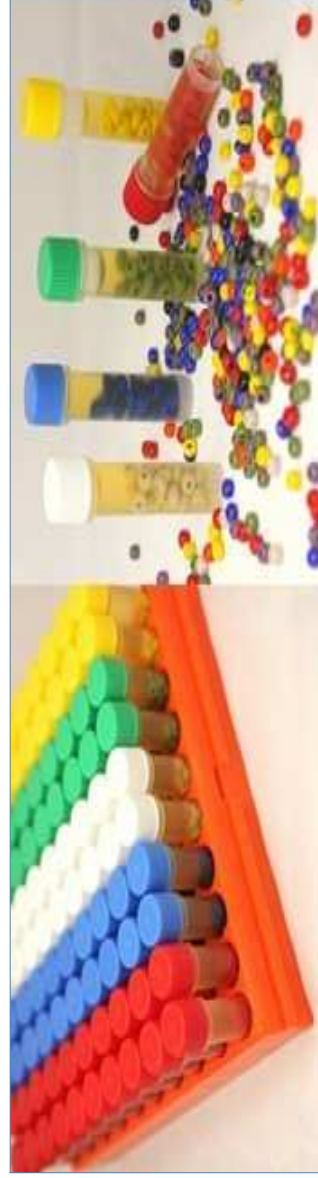
- means negative result, + means positive result.



**Fig. 1** A representative RT-PCR for Beg9/End9 primers (1,062 bp).



**Fig. 2** A representative RT-PCR for Con2/Con3 primers (880 bp).



# Molecular BioSystems

Cite this: *Mol. BioSyst.*, 2012, **8**, 1060–1067

[www.rsc.org/molecularbiosystems](http://www.rsc.org/molecularbiosystems)

## Comparative proteomics to evaluate multi drug resistance in *Escherichia coli*†

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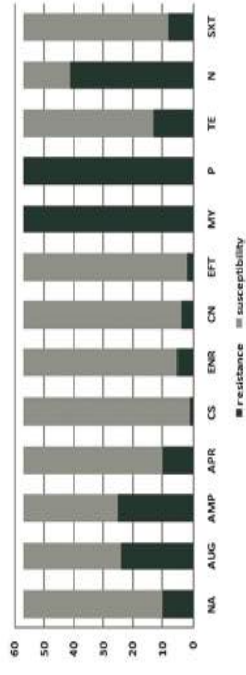
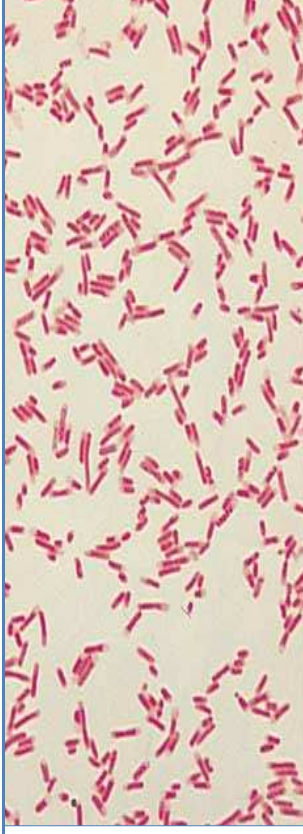


Fig. 1 Number of bacteria resistant to each tested antibiotic, considering 57 isolates. NA: nalidixic acid, AUG: augmentin, AMP: ampicillin, APR: apramycin, CS: colistin sulfate, ENR: enrofloxacin, CN: gentamicin, EFT: ceftiofur, MY: lincomycin, P: penicillin, TE: tetracycline, N: neomycine, SXT: sulfamethoxazole/trimethoprim.

Drug resistance in food-borne bacterial pathogens is an almost inevitable consequence of antimicrobial drugs, used either therapeutically or to avoid infections in food animals. In the past decades, the spread and inappropriate use of antibiotics have caused a considerable increase of antibiotics to which bacteria have developed resistance and bacteria are becoming resistant to more than one antibiotic simultaneously. Understanding the mechanisms at the molecular level is extremely important to control multi-resistant bacteria and to develop new therapeutic strategies. In the present study, comparative proteomics characterized membrane and cytosolic proteome in order to investigate the regulation of expression in multi-resistant *E. coli* isolated from young never vaccinated water buffalo. This study highlighted differentially expressed proteins under multi drug resistance conditions and provided insights about mechanisms involved in resistance, as quorum sensing mechanism and other signaling pathways. These findings suggest possible novel bacterial targets to develop alternative antibiotic drugs.

Table 1 Antibiotic resistance profile related to samples included in this study. 'R' means resistance to the tested antibiotic. On the back of the resistance/susceptibility test, samples were classified into C and R groups. C groups: samples 1, 2, 6, 10, 11, and 12 (resistant to a maximum of 1 antibiotic, excluding MY, P and N, common to all R groups); R groups: 3, 4, 5, 6, 7, and 9.

Sample	NA	AUG	AMP	APR	CS	ENR	CN	EFT	MY	P	TE	N	SXT
1	R	R	R	R	R	R	R	R	R	R	R	R	R
2	R	R	R	R	R	R	R	R	R	R	R	R	R
3	R	R	R	R	R	R	R	R	R	R	R	R	R
4	R	R	R	R	R	R	R	R	R	R	R	R	R
5	R	R	R	R	R	R	R	R	R	R	R	R	R
6	R	R	R	R	R	R	R	R	R	R	R	R	R
7	R	R	R	R	R	R	R	R	R	R	R	R	R
8	R	R	R	R	R	R	R	R	R	R	R	R	R
9	R	R	R	R	R	R	R	R	R	R	R	R	R
10	R	R	R	R	R	R	R	R	R	R	R	R	R
11	R	R	R	R	R	R	R	R	R	R	R	R	R
12	R	R	R	R	R	R	R	R	R	R	R	R	R



## Case Report

# *Streptococcus constellatus*-associated pyoderma in a dog

Luisa De Martino,<sup>1</sup> Sandra Nizza,<sup>1</sup> Claudio de Martinis,<sup>1</sup> Valentina Foglia Manzillo,<sup>2</sup> Valentina Iovane,<sup>1</sup> Orlando Paciello<sup>1</sup> and Ugo Pagnini<sup>1</sup>

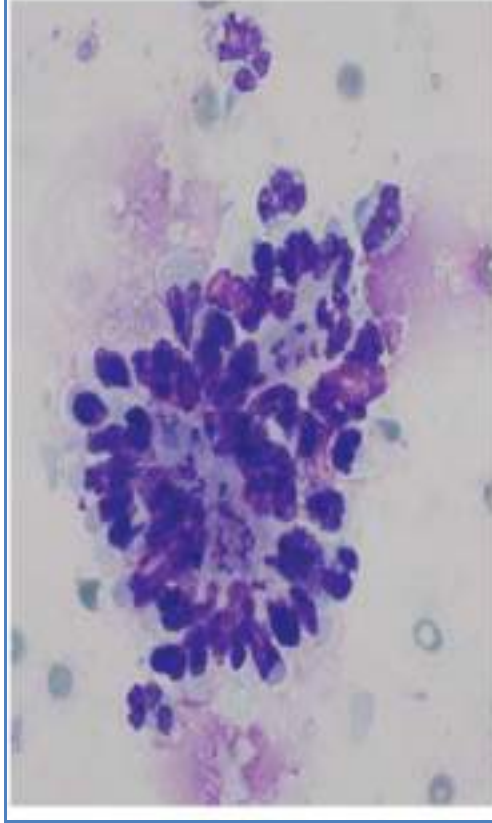
This report describes a case of chronic and deep pyodermitis in a 4-year-old male dog with a 3-month skin problems history that had been treated unsuccessfully with fluoroquinolone therapy, prescribed by a private medical veterinary practice, without an early diagnosis. Microbiological examination and antimicrobial susceptibility testing were performed in our laboratory (Faculty of Veterinary Medicine) and a diagnosis of *Streptococcus constellatus*-associated pyoderma in the dog was made. A new antimicrobial treatment, with tetracyclines, was designed after the definitive diagnosis and antimicrobial susceptibility testing performed by the Kirby–Bauer disc diffusion method. The dog remained free of clinical illness at completion of therapy. To our knowledge, this is the first case of a canine pyoderma caused by *S. constellatus*, a commensal organism which may also cause pyogenic infections. Furthermore, this study confirms that a fluoroquinolone represents a poor empirical choice for initial therapy of canine pyoderma.



**Fig. 1.** Large nodular lesions on the lumbar region with purulent exudates are present.



**Fig. 3.** Photomicrograph of a cytological slide showing several degenerated neutrophils associated with many spherical-shaped bacteria.



## Penicillin-Resistant *Aerococcus viridans* Bacteremia Associated with Bovine Severe Respiratory Syndrome

Jacopo Guccione<sup>1</sup>, Sandra Nizza<sup>2</sup>, Karina Mallardo<sup>2</sup>, Antonietta Cantello<sup>2</sup>, Filomena Fiorito<sup>2</sup>,  
Antonio Di Loria<sup>1</sup>, Luisa De Martino<sup>2\*</sup>

### ABSTRACT

*Aerococcus viridans*, a less frequently isolated bacteria, is a gram-positive, catalase-negative coccus, found singly or in tetrads, with biochemical and growth characteristics of streptococci and enterococci. This microorganism, usually susceptible to penicillin, is often found in the environment and is infrequently associated with human/veterinary infections. We described a case of Holstein Friesian female calf, 150-day-old, affected by respiratory emergencies. Following the clinical signs, radiographic analysis and bacteriological/molecular examinations carried out on blood culture, a diagnosis of severe broncho-pulmonary disease associate with a multidrug-resistant *A. viridans* bacteremia was done. The present case highlights the invasive nature of a saprophytic bacterium showing a broad profile of antibiotic-resistance including  $\beta$ -lactams. Furthermore, this report confirms that the effectiveness of an antibiotic therapy is based primarily on a sure diagnosis including susceptibility testing.



Table 1. Susceptibility of the *A. viridans* strains to sixteen antibiotics.

Category	Antibiotics	Susceptible/Intermediate
Penicillins	Penicillin	R
	Amoxicillin and Clavulanic	R
	Ampicillin	R
Aminoglycosides	Amikacin	R
	Gentamicin	R
	Kanamycin	I
	Ceftazidime	R
	Carbapenem	R
Cephalosporins	Ceftriaxone	R
	Cefepime	R
Fluoroquinolones	Enrofloxacin	R
	Erythromycin	S
	Telavancin	S
Macrolides	Erythromycin	S
	Telavancin	S
Sulfonamides + Diaminopyrimidines	Trimethoprim-Sulfamethoxazole	R
	Tetraacyclines	S
Tetracyclines	Tetracycline	S
	Doxycycline	S

S = Susceptible, I = Intermediate, R = Resistant (All antibiotic doses are from Otsuka Ltd., except telavancin from Santis-Crista bio)





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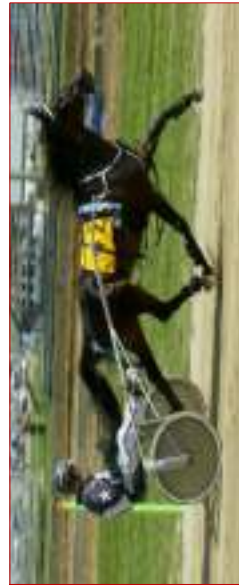
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## A comparative evaluation of methicillin-resistant staphylococci isolated from harness racing-horses, breeding mares and riding-horses in Italy

Karina Mallardo, Sandra Nizza, Filomena Fiorito, Ugo Paggini, Luisa De Martino\*



### ABSTRACT

**Objective:** To investigate the prevalence of methicillin-resistant staphylococci (MRS), which is a potential risk factor of transmission between animals and humans in different types of horses (harness racing-horses, breeding mares and riding-horses) and to compare the antimicrobial resistance of the isolates. **Methods:** A total of 191 healthy horses, housed at different locations of the Campania Region (Italy), were included in the study. Nasal swab samples were collected from each nostril of the horses. The *mecA* gene was detected by a nested PCR technique. Antibiotic susceptibility was tested for each isolate. **Results:** MRS was isolated from nasal samples of 68/191 (35.6%; 95% CI: 28.9%-42.9%) healthy horses. All isolates were coagulase-negative with the exception of two coagulase-positive MRS strains, identified as *Staphylococcus aureus* and *Staphylococcus pseudintermedius*, 2/83 (2.4%; 95% CI: 0.4%-9.2%). Interestingly, both coagulase-positive MRS isolates were from harness racing-horses. These horses also presented a significantly higher positivity for MRS (53.3%; 95% CI: 40.1%-66.1%) than the breeding mares and riding-horses groups. Antibiotic susceptibility testing showed difference between isolates due to different origins except for an almost common high resistance to aminopenicillins, such as ampicillin and amoxicillin. **Conclusions:** It can be concluded that harness racing-horses may act as a significant reservoir of MRS as compared to breeding mares and riding-horses.

**Table 1**  
Distribution of MRS isolated from nasal swabs of horses.

Horse type	Number of horses	Number of isolates	Positive horses (%) CI (95)	MRS
Harness racing horses	80	37	46.3-66.1	<i>S. sciuri</i> (2); <i>S. lentus</i> (1); <i>S. sapientis</i> (5); <i>S. xylosum</i> (4); <i>S. aureus</i> (1); <i>S. maricandara</i> (1); <i>S. zoochalis colvati</i> (1); <i>S. Avonius</i> (1); <i>S. pseudintermedius</i> (1)
Breeding mares	64	20	26.6-50.3	<i>S. lentus</i> (24); <i>S. xylosum</i> (4); <i>S. sciuri</i> (2)
Riding horses	67	16	18.1-26.6	<i>S. sciuri</i> (1); <i>S. xylosum</i> (1); <i>S. lentus</i> (1)

By the Student-Newman-Keuls Multiple Comparisons test, the prevalence of MRS was significantly different between the three groups of the examined horses. Number in the bracket indicates the number of isolates for each *Staphylococcus* species.



**Table 2**

Percentage of resistance to 18 antimicrobial agents

Antibiotics	Harness racing-horses		Breeding mares	
	<i>S. lentus</i>	<i>S. sciuri</i>	<i>S. lentus</i>	<i>S. xylosum</i>
OX	100.0	100.0	100.0	100.0
AMC	100.0	100.0	100.0	33.3
AMP	100.0	100.0	100.0	8.3
IMI	0.0	0.0	0.0	16.6
MRP	0.0	0.0	0.0	75.0
CEC	0.0	0.0	0.0	41.6
CXM	0.0	0.0	0.0	66.6
CPR	0.0	0.0	0.0	66.6
CRO	9.1	0.0	0.0	75.0
FOX	9.1	0.0	0.0	66.6
E	18.2	25.0	25.0	25.0
MY	100.0	100.0	100.0	58.3
TE	72.7	25.0	25.0	41.6
DXT	72.7	25.0	25.0	41.6
AK	18.2	8.3	0.0	0.0
SXT	0.0	0.0	0.0	0.0
CS	0.0	0.0	0.0	25.0
CIP	27.3	8.3	0.0	25.0

OX: oxacillin; AMC: amoxicillin/clavulonic acid; IMI: imipenem; MRP: meropenem; CEC: cefaclor; CPR: cefprozil; CRO: ceftriaxone; FOX: cefoxitin; lincomycin; TE: tetracycline; DXT: doxycycline; trimethoprim/sulphamethoxazole; CS: colistin sul



**A successful vancomycin treatment of  
multidrug-resistant MRSA-associated canine pyoderma**

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80137 Naples, Italy.

**Case report.** This report describes a case of diffuse pyoderma in a 10-year-old female dog with hypothyroidism. A previous treatment, without an early diagnosis, including cephalosporin associated with prednisolon resulted to be unsuccessfully. After clinical and microbiological examination in our laboratories, a diagnosis of methicillinresistant *Staphylococcus aureus* (MRSA)-associated pyoderma was made. The antimicrobial susceptibility testing evidenced many resistances and susceptibility of the strain only to vancomycin and linezolid. A new therapy against hypothyroidism and associated with an appropriate antimicrobial (vancomycin) treatment, improved and resolved the infection.

**Clinical significance.** To our knowledge, this is the first case of canine pyoderma caused by a strain of MRSA with a such severe multiresistant profile. MRSA infections present a serious challenge because of the emergence of resistance to numerous conventional antibiotics and the risk factors associated with the transfer of the bacteria to humans, who have a contact with infected pets.



Fig. 1 - A 10-year-old female Fila Brasileiro with alopecia, erythema, erosions, ulcers



Fig. 2 - Dog improvement after specific treatment



# salmonellosi

## Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region – Italy

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**Table 2**

Distribution of *Salmonella* among European wild boars of different sexes and ages that were shot during the 2010–2011 and 2011–2012 in the Natural Park Aurunci in the Latium region.

Salmonella spp.	N. of positive animals of different sexes				N. of positive animals of different y				
	N. of positive animals/samples studied	Male	Female	UNK	<1	1–2	2–3	3–4	4–
Microbiological assay	54/499	25	23	6	8	23	11	7	0
Serological assay	255/383	122	128	5	19	58	42	60	39

UNK, unknown.

**Table 4**

Occurrence of antimicrobial drug resistance in 54 *Salmonella* spp. isolates from 499 European wild boars in the Latium region.

No. of Strains	% of resistance to																% of resistance to drugs				
	A	AC	Cz	CEf	Crx	S	Gm	N	K	Su	SXT	Nx	Eno	C	T	Cl	0	1	2	3	≥4
54	3.7	1.8	1.8	3.7	1.8	18.5	5.5	0	0	92.5	14.8	1.8	0	0	5.5	14.8	7.4	42.5	33.3	11.1	5.5

**Abbreviations.** Ampicillin (A, 10 µg); amoxicillin and clavulanic acid (AC, 30 µg); cefazoline (Cz, 30 µg); cefotaxime (Crx, 30 µg); ceftiofur (CEf, 30 µg); streptomycin (S, 10 µg); gentamycin (Gm, 10 µg); neomycin (N, 30 µg); kanamycin (K, 30 µg); sulphamethoxazole (Su, 300 µg); trimethoprim–sulphamethoxazole (SXT, 23.75/1.25 µg); nalidixic acid (Nx, 30 µg); enrofloxacin (Eno, 5 µg); chloramphenicol (C, 30 µg); tetracycline (T, 30 µg); and colistin (Cl, 10 µg).



## Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region – Italy

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**Table 3**

*Salmonella* serovars or subspecies isolated from 499 European wild boars in the Latium region.

Serovars	Group	Antigenic formula	Number
<i>S. enterica</i> subsp. <i>salamae</i> II	O:42 (T)	42:z:1,5	9 (16.6%)
	O:41 (S)	41:z:1,5	4 (7.4%)
<i>S. enterica</i> subsp. <i>Diarizonae</i> III b	O:50 (Z)	50:r:1,5,7	3 (5.5%)
	O:61	61:c:z35	1 (1.8%)
	O:61	61:i:e,n,x,z15	2 (3.7%)
	O:17 (J)	17:z10:e,n,x,z15	2 (3.7%)
	O:38 (P)	38:z4,z23:-	2 (3.7%)
<i>S. enterica</i> subsp. <i>houstenae</i> IV	O:40 (R)	40:z4,z23:-	3 (5.5%)
	O:43 (U)	43:z4,z23:-	1 (1.8%)
	O:16 (I)	16:a:1,5	
<i>S. Fischerhuette</i>	O:11 (F)	11:i:e,n,x	
<i>S. Veneziana</i>	O:9 (D1)	9:12:Lz13:e,n,x	
<i>S. Napoli</i>	O:8 (C2–C3)	6,8:e,h:1,5	
<i>S. Kottbus</i>	O:7 (C1)	6,7:k:1,5	
<i>S. Thompson</i>	O:48 (Y)	48:z4,z23:-	
<i>S. enterica</i> subsp. <i>arizonae</i> III a	O:18 (K)	18:Lw'e:n,z15	
<i>S. Toulon</i>	O:4 (B)	4,5,12:i:1,2	
<i>S. Typhimurium</i>	O:4 (B)	4,5,12:i:-	
<i>S. enterica</i> subsp. <i>enterica</i>	O:4 (B)	4,5,12:z4,z23:-	
<i>S. Stanleyville</i>	O:16 (I)	16:Lv'e:n,z15	
<i>S. Burgas</i>	O:18 (K)	18:r:1,5	
<i>S. Tennebone</i>	O:8 (C2–C3)	8:e,h:1,5	
<i>S. Ferruch</i>	O:40 (R)	40:z4,z23	
<i>S. Choleraesuis</i>	O:4 (B)	4,5,12:b:1,2	
<i>S. Paratyphi</i>			



## Prevalence and antimicrobial susceptibility of *Salmonella* in European wild boar (*Sus scrofa*); Latium Region – Italy

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# salmonellosi

**Table 5**

Occurrence of antimicrobial drug resistance profiles in 54 *Salmonella* spp. isolates from 499 wild boars in the Latium region.

Resistance profile	No. (%) of isolates with the given resistance profile
A; S; SU; SXT; T	1 (2)
A; AC; SU; SXT	1 (2)
CEF; CTX; S; SU	1 (2)
Cz; SU; SXT	1 (2)
GM; SU; CL	1 (2)
GM; SU; T	1 (2)
S; SU; CL	1 (2)
S; SU; SXT	1 (2)
SU; Nx; T	1 (2)
CEF; SU	1 (2)
GM; SU	1 (2)
SU; T	1 (2)
SU; SXT	4 (8)
SU; CL	5 (10)
S; SU	6 (12)
SU	23 (46)

**Abbreviations.** Ampicillin (A); amoxicillin and clavulanic acid (AC); cefazoline (Cz); cefotaxime (CTX); ceftiofur (CEF); streptomycin (S); gentamycin (GM); neomycin (N); kanamycin (K); sulphamethoxazole (SU); trimethoprim–sulphamethoxazole (SXT); nalidixic acid (Nx); enrofloxacin (ENO); chloramphenicol (C); tetracycline (T); and colistin (CL).

# Residui di inibenti e antibiotico resistenza in prodotti ittici pescati nel golfo di Salerno

Poster pubblicato ed esposto all'ARIP (2nd Conferenza Europea sull'antibiotico-resistenza) Vilnius, **Lituania** Ottobre 2012

In collaborazione con il Dipartimento di **Ispezione** della Facoltà di Medicina Veterinaria



**Presence of antibiotic residues and occurrence of antibiotic resistance in bacteria isolated from seawater fish and fishery products**

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in corso di pubblicazione su  
BMC Veterinary Research



Porto Masuccio Salernitano

# Risultati della ricerca

- Residui di Ab in polipo e seppia (dimostra il rilascio di sostanze inibenti nelle acque reflue e quindi nel mare)
- Resistenza batterica ai glicopeptidi (antibiotici di uso crescente negli ospedali) da parte di *Vibrio alginolyticus* e *parahaemolyticus*
- Ceppi resistenti isolati in sgombro e calamaro

N.B. La AR è più pericolosa della presenza di Ab perché può essere trasmessa da animale all'uomo e viceversa (Fonte FAO/OIE/WHO)





# PRESENCE OF RESIDUES OF ANTIMICROBIAL SUBSTANCES AND ANTIMICROBIAL RESISTANCE IN FISH CAUGHT IN GULF OF SALERNO, CAMPANIA REGION, SOUTH ITALY



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## Introduction

Drug resistance is a global public health problem, which is exacerbated by the widespread use of antimicrobials in human and animal health. The overuse of antimicrobials in human and animal health is a major public health problem, which is exacerbated by the widespread use of antimicrobials in human and animal health. The overuse of antimicrobials in human and animal health is a major public health problem, which is exacerbated by the widespread use of antimicrobials in human and animal health. The overuse of antimicrobials in human and animal health is a major public health problem, which is exacerbated by the widespread use of antimicrobials in human and animal health.

## Aim of the work

The aim of this work was to investigate the presence of antimicrobial residues in fishery products using a screening procedure that was able to detect the presence of antimicrobial residues in a wide range of fish species.



## Materials and Methods

During the study, 100 fish samples were collected from 10 different fish species in the Gulf of Salerno (Campania Region, South Italy) in two different periods (July and June 2022) with the aim of investigating the presence of antimicrobial residues in fishery products. The samples were collected from 10 different fish species in the Gulf of Salerno (Campania Region, South Italy) in two different periods (July and June 2022) with the aim of investigating the presence of antimicrobial residues in fishery products. The samples were collected from 10 different fish species in the Gulf of Salerno (Campania Region, South Italy) in two different periods (July and June 2022) with the aim of investigating the presence of antimicrobial residues in fishery products.

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## Results

The results of the screening procedure showed the presence of antimicrobial residues in 10% of the samples collected. The most common residues detected were tetracyclines, followed by penicillins and sulfonamides. The results of the screening procedure showed the presence of antimicrobial residues in 10% of the samples collected. The most common residues detected were tetracyclines, followed by penicillins and sulfonamides. The results of the screening procedure showed the presence of antimicrobial residues in 10% of the samples collected. The most common residues detected were tetracyclines, followed by penicillins and sulfonamides.

## Conclusions

The presence of antimicrobial residues in fishery products is a public health concern. The results of this study show that antimicrobial residues are present in 10% of the samples collected. The most common residues detected were tetracyclines, followed by penicillins and sulfonamides. The results of this study show that antimicrobial residues are present in 10% of the samples collected. The most common residues detected were tetracyclines, followed by penicillins and sulfonamides.



Fig. 1



Table 1. Antimicrobial resistance patterns in fishery products.

1. Scalabrino C, Marotta R, Altieri G, Di Ciccio S, Cappabianco S, Scuderi P, Anartzano A. (2023) Presence of antimicrobial residues and antimicrobial resistance in fish caught in the Gulf of Salerno, Campania Region, South Italy. *Antimicrob. Resist. Infect. Control* 18: 1-10. doi:10.1017/S1750268823000001

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## Presence of antibiotic residues and occurrence of antibiotic resistance in bacteria isolated from seawater fish and fishery products

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Different seawater fish and fishery products was examined to detect antibiotic residues and to evaluate the presence of several *Vibrio* strains. In addition bacteria isolated, were successively examined for the incidence of antibiotic resistance. Three antibiotic substances (erythromycin, sulfamethoxazole and trimethoprim) were detected (by screening and confirmatory methods) in Octopus, Cuttlefish and Murex. All *Vibrio* strains isolated from fish were resistant to Teicoplanin (TEC) and Vancomycin (VA). In *Vibrio alginolyticus*, isolated in octopus, a resistance against 9 antibiotics was noted.

### 1. Introduction

Environmental contamination by pharmaceuticals is an important issue. Medical substances may roughly be divided into medical substances used by human or veterinary medicine. The veterinary drugs may further be subdivided into substances used as growth promoters for livestock production, therapeutics in livestock productions, coccidiostatic used for poultry production, therapeutics for treatment of livestock on fields or as feed additives in fish farms.

Drugs administered to humans and animals are excreted with urine or faeces [1] and attend the sewage treatment plant (where present) [2,3]; successively if substances are hydrophilic or are metabolized to a more hydrophilic form of the parent lipophilic substance, will pass the waste water treatment plant and end up in the receiving waters (waste water treatment effluents often discharge to rivers) where they may be are present at very low concentrations but active ingredients are designed to stimulate a response in humans and animals at low doses with a very specific target [4], making the implications for human health and the environment a matter of concern. A recent study showed that a mixture of drugs at the concentrations actually found in the aquatic environment of some Italian areas is able to exert toxic effects on the proliferation of human and zebra fish cells cultures [5]. The main consequences of abuse of antibiotics is the development and diffusion of antibiotic resistance that represent a public health problem, with obvious consequences in human and veterinary medicine, since it affects animal therapy and food safety [6]. The growing alarm related to the spreading of the resistance of antibiotics considered of first choice in the treatment of specific human infections prompted measures for antimicrobial resistance surveillance of bacteria circulating in humans, animals and food products.

Aim of this study was to evaluate the presence of antimicrobial substances and to assess the antimicrobial resistance in bacteria species isolated from seawater fish and fishery products caught in Campania region (southern Italy).

### 2. Materials and Methods

Samples were collected in the gulf of Salerno (Campania Region, Southern Italy) with the support of the mobile station of the Fish Research Laboratory of the Department of Veterinary Medicine and Animal production, University of Naples "Federico II".

L'ampio utilizzo della terapia antibiotica empirica-ragionata, non prevede l'ausilio del laboratorio di Batteriologia, quindi non fornisce alcuna indicazione sull'agente batterico responsabile dell'infezione e tantomeno sulla sensibilità di quest'ultimo ai diversi antibiotici.

### **Nostro obiettivo:**

Definire l'andamento delle resistenze alle classi di antibiotici più frequentemente prescritti dai medici veterinari sul territorio





# III WORKSHOP DELLA MICROBIOLOGIA VETERINARIA DI NAPOLI La microbiologia: saperi nel mondo

25 Maggio 2017

Aula Magna Orto Botanico, Via Faria 223 - Napoli

8.00 Registrazione dei partecipanti

9.15 Presentazione della giornata

Giuseppe Iovene, Luca De Martino

## Indirizzi di salute

Giuseppe Altarelli - Rettore dell'Università degli Studi di Napoli "Federico II"

Giuseppe Olivero - Direttore del Dipartimento di Medicina Veterinaria e Produzioni Animali

Anna Teresa Palombara - Presidente della Società Italiana di Microbiologia (SIM)

Antonio Limone - Direttore Generale Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM)

Paolo Samioli - Responsabile Settore Veterinario Regione Campania

## PRIMA SESSIONE: Stafilococchi e Antibiotici

10.00-11.30

Moderatori: Giuseppe Iovene, Patrizia Nebbia

Luca Guarnabassi - "Antibiotico-resistenza in *Staphylococcus pseudintermedius*: una crescente

minaccia per la salute dei piccoli animali da compagnia"

Stefania Stefani - "S. aureus umani e animali: sono tutti potenzialmente stamif"

Discussione

Pausa caffè

## SECONDA SESSIONE: Interferenze degli Antibiotici

12.00-13.20

Moderatori: Alessandro Benetti, Vincenzo Cuteri

Giorgio Galloro - Effetti degli antibiotici sul microbiota intestinale

Adriano Vignoli - Biofilm e antibiotico-resistenza

Discussione

Pausa pranzo 13.30 - 14.30

## TERZA SESSIONE : Comunicazioni brevi

14.30-15.45

Moderatori: Maria Tempesta, Maria Cristina Di Giovanni, Mazzatullo Galdero

Piera Anna Martello - "Resistenza alla mectidina e praziquantel di ceppi di *Strongylus edentatus* da campioni di origine canina"

Blanca Maria Orlandella - "Isolamento di *Staphylococcus aureus* da campioni di origine canina"

Patrizia Rebbio - "Caratterizzazione genetica di ceppi di *Escherichia coli*"

Mariacristina Correnti - "Fattori nutrizionali in associazione con l'antibiotico-resistenza canina e equina"

Gianni Pizzotto - "Prevalenza di microrganismi antibiotico-resistenti scoperti da animali selvatici in trasversali italiane"

Paola Motta - "Efficacia di antibiotico-resistenza nel cane"

Luca De Martino - "Open...reporting"

Discussione finale e chiusura lavori

Comitato scientifico: L. De Martinis, G. Iovene, G. Iovanna, D. Pagani, S. Romagnolo. Tel. 081-5241160. [info@sim.it](mailto:info@sim.it)

Numero verde di partecipazione: 100 - [www.sim.it](http://www.sim.it)



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grazie