ÉCOLE PRATIQUE des HAUTES ÉTUDES | PSL

Antibiotics and others pharmaceuticals in the environment

Introduction

- Human activities generate numerous environmental disturbances:
  - Biological
  - Physical: Heat, Suspended solids (SS), Radioactivity
  - Chemical: Metals, Organic molecules (nutrients, micropollutants)

- Contaminant ≠ pollutant

  - Chapman, Env. Int., 2007

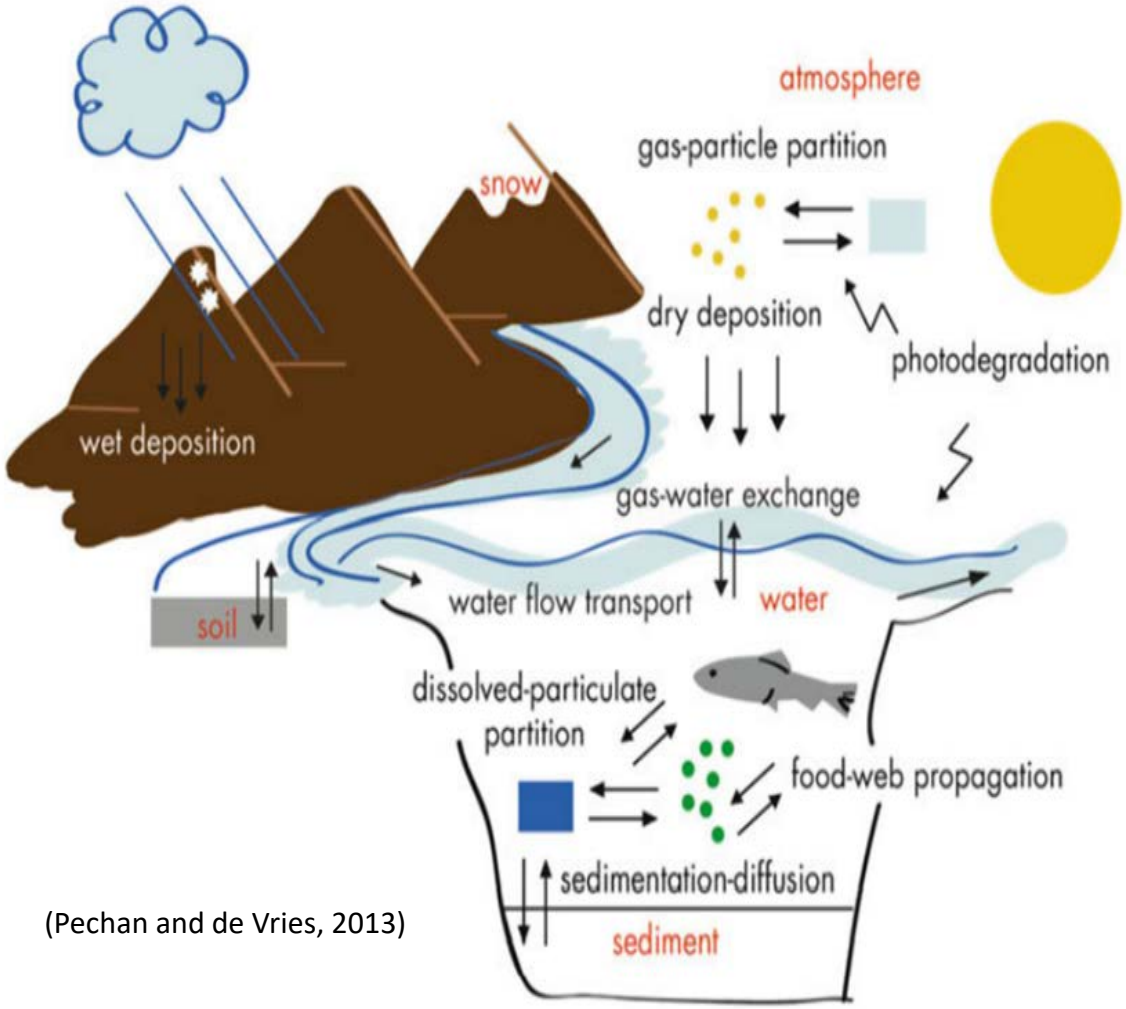
  « Contamination is simply the presence of a substance where it should not be or at concentrations above background. Pollution is contamination that results in or can result in adverse biological effects to resident communities. All pollutants are contaminants, but not all contaminants are pollutants. »

Micropollutants

- Lists of hazardous substances (1976) or WFD priority substances (2000) → Families of molecules
  - Different behaviours within the same family
  - Indicators of sources and dispersion modes
  - Regulatory developments Substitute molecules
- Pesticides : DDT, triazines, organophosphates
- Persistants organic pollutants (POP) : PCBs, HCB…
- Accidental synthesis products : Dioxins, PAHs…
- Plasticizers : phthalates, BPA…
- Flame retardants: PBDEs, TBBPA…
- Surfactants : alkylphenols

- « Undesirable molecules"
  - Drugs: antibiotics (sulphonamides, tetracyclines, fluoroquinolones, etc) and others pharmaceuticals
  - Estrogens (hormones) : 17α et 17β estradiol
  - Preservatives (parabens, triclosan, etc…)
  - Synthetic musk (galaxolide, tonalide, etc)

Pathways of diffusion of micropollutants in the environment



(Pechan and de Vries, 2013)

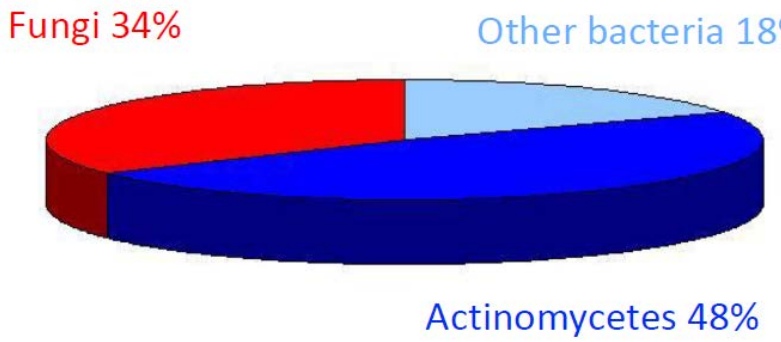
What about Antibiotics ?
And their adserve effects ?

Outline :

- History of Antibiotics (ATB)
- Uses of ATB
- Development of Antimicrobial resistance (AMR)
- Rôle of environmental Pressure
- How ATB enter in environment
- Levels of ATB concentrations in environment
- Implications for wildlife
- Others pharmaceuticals

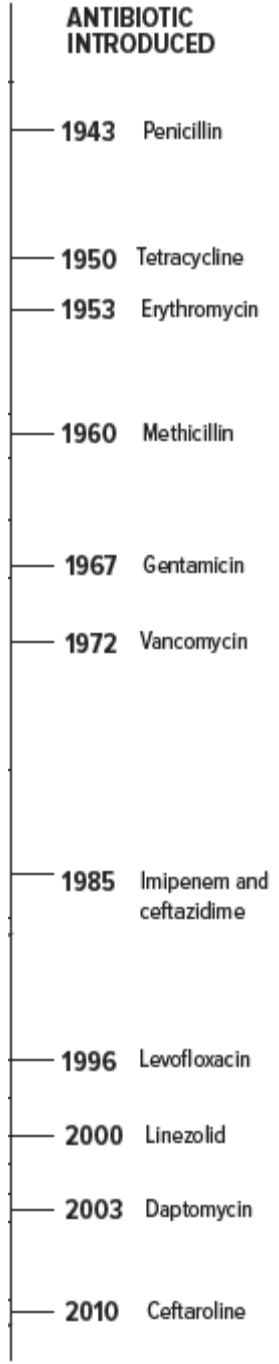
History of antibiotics

- History
  - Penicillin (Fleming 1928/Chain &Florey 1939/ treat. 1941)
  - Sulfonamide (Dogmak 1931 / Trefouel 1935)
  - 1940 actinomycin; 1944 streptomycin
  - 1946: industrialisation and marketing



- Origins

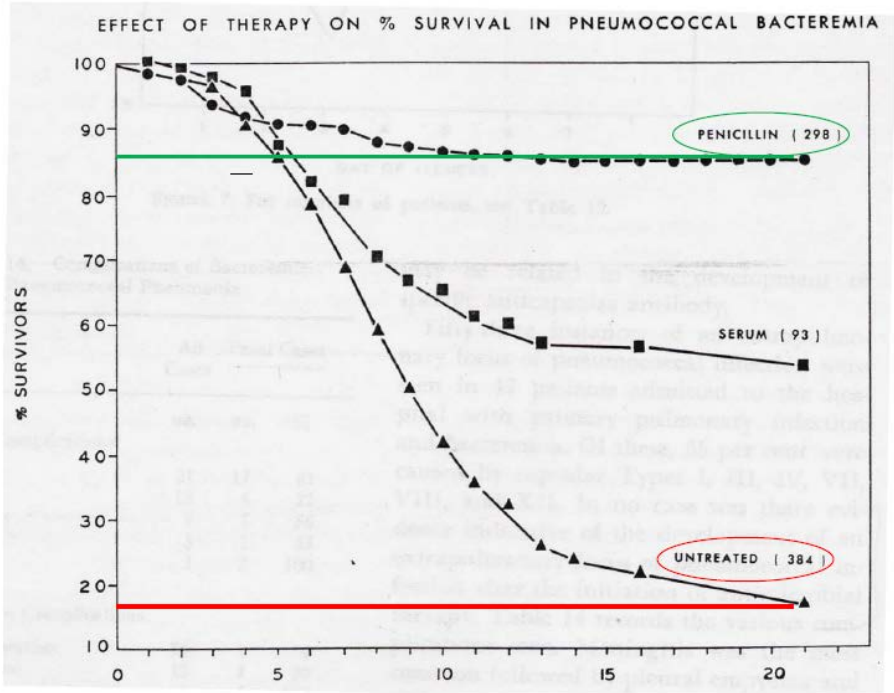
  Most current antibiotics are either natural molecules produced by bacteria (mostly actinomycetes) and fungi, that can be modified chemically, or fully synthetic (sulfonamide and trimethoprime)



A major tool of modern medicine

- The miracle of antibiotics
  - Save life of soldiers (WW2)
  - ⇗ % survival during bacterial infection
  - saved millions of lives
  - Between 1944 and 1972 human life expectancy jumped by eight years

- Uses
  - Bacteria
  - Surgery
  - Oncology
  - transplantation



Austrian R and Gold J, Ann of Intern Med 1964

A major tool for veterinary medicine

- mostly deals with groups of animals (livestock)
- treat dogs, cats, horses individually

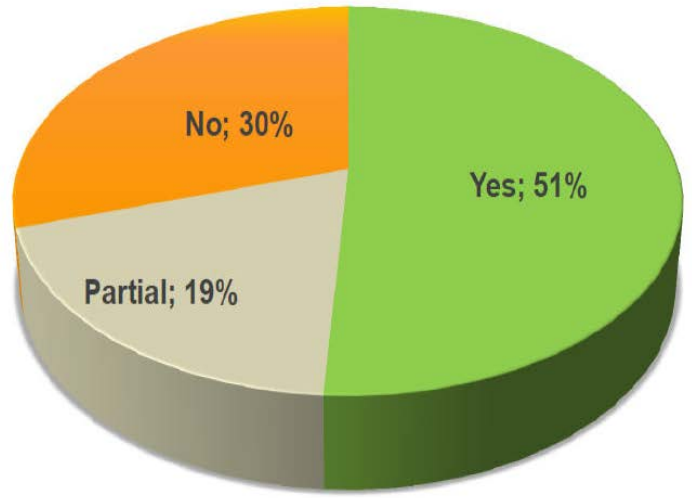
- Uses
  - as growth promoters

    49% OIE/WOAH members countries

    In Europe, Use banned in 2006

  - therapeutic use (with a marketing authorization or not)

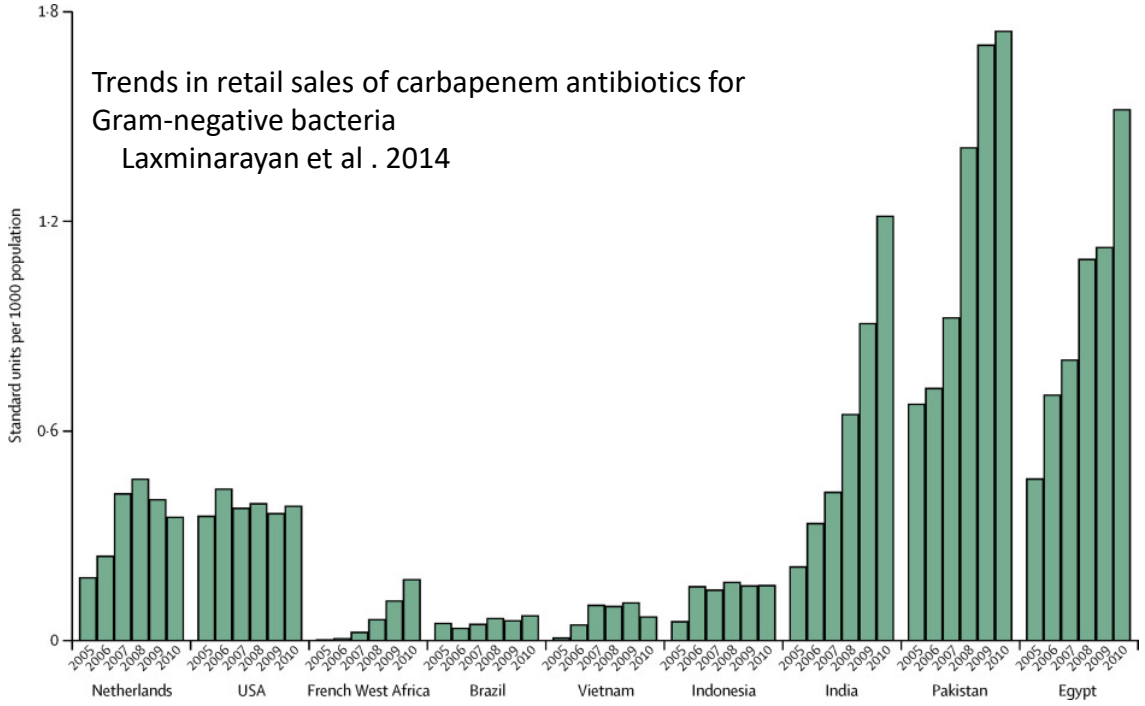
Proportion of OIE Member Countries banning the use of antimicrobial agents as growth promoters



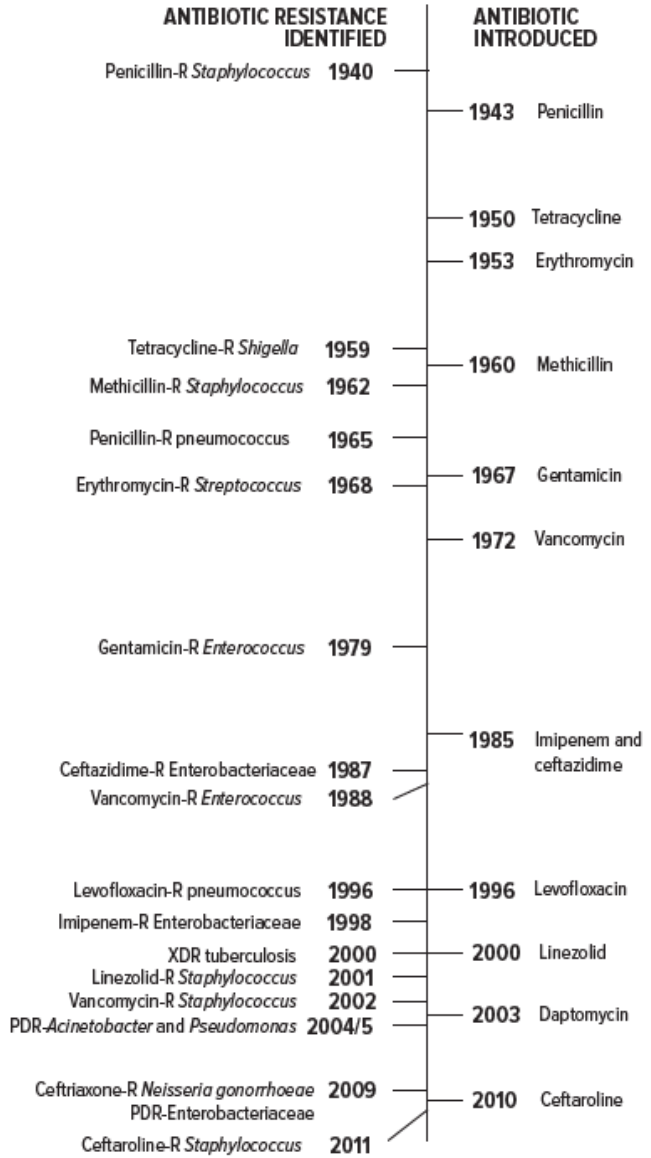
152 questionnaires received from 178 OIE Member Countries = **85% replied**.

Development of Antimicrobial resistance

- Natural processus since -3,8 billion years (Bacteria) as defensive mechanism
- Increase uses (humans, animals) favours the development of Antimicrobial resistance (AMR)



Trends in retail sales of carbapenem antibiotics for Gram-negative bacteria
Laxminarayan et al . 2014



Ventola, 2015

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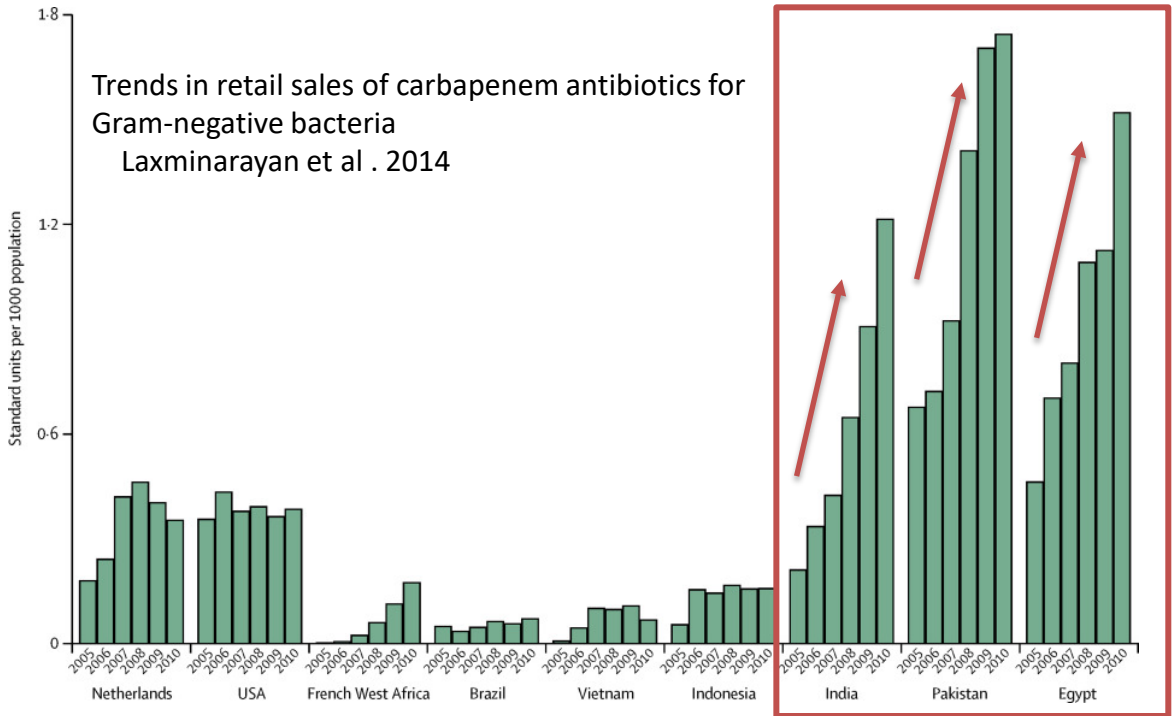
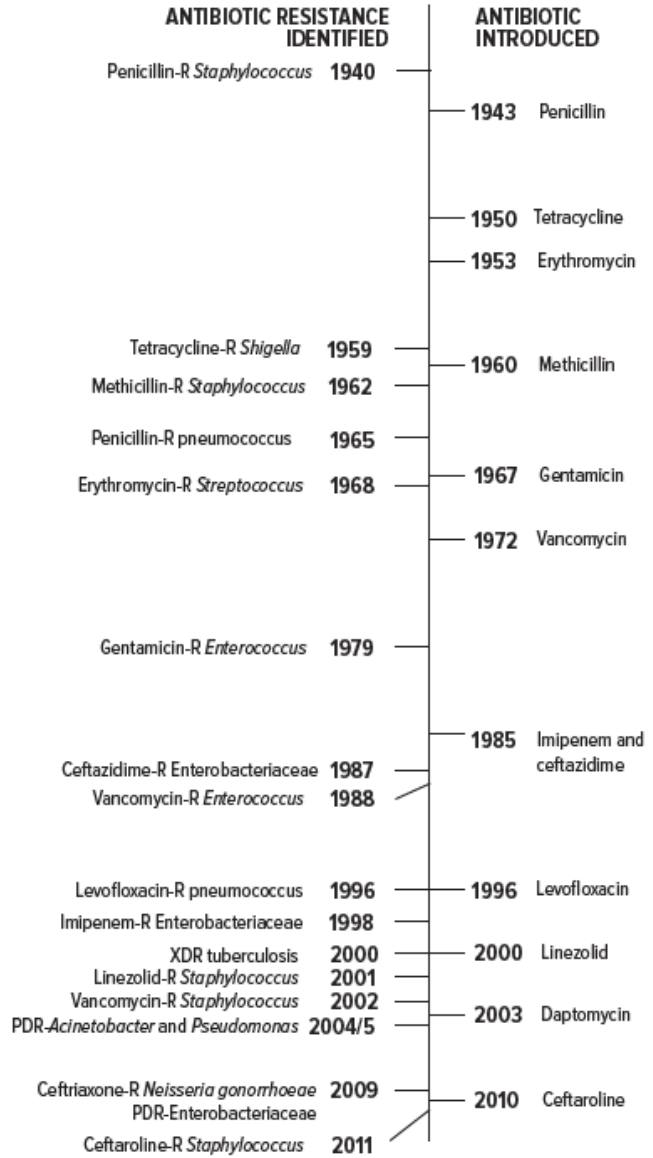
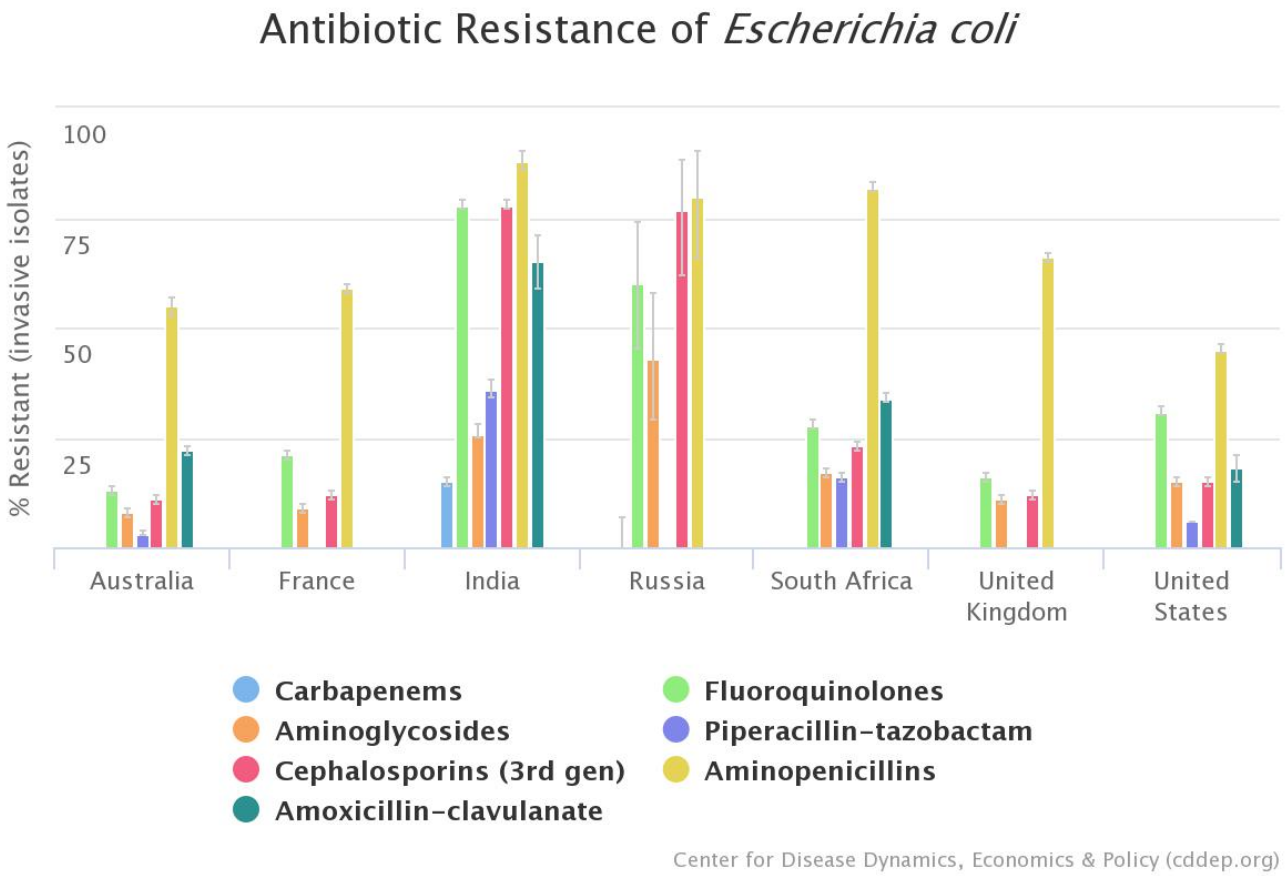




Ventola, 2015

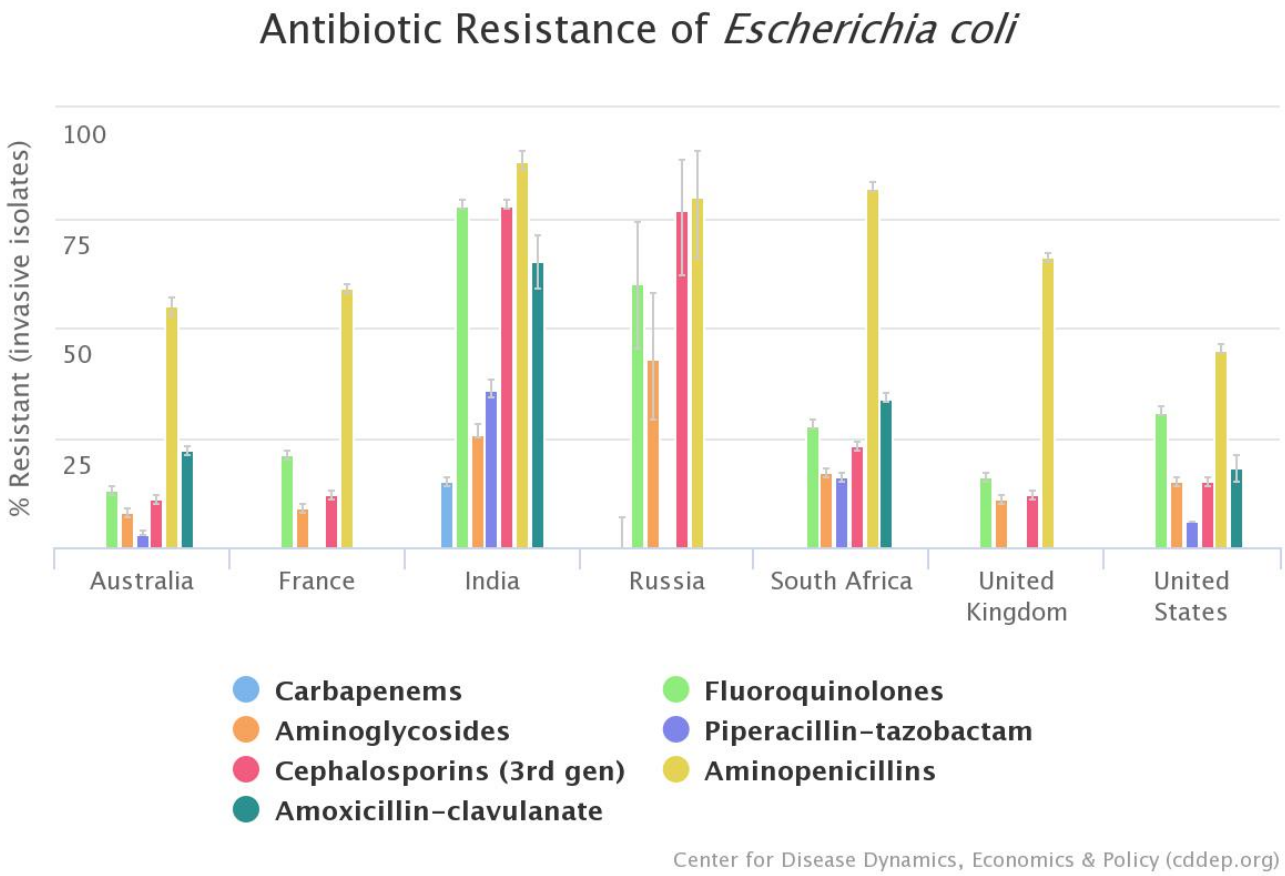
Development of Antimicrobial resistance



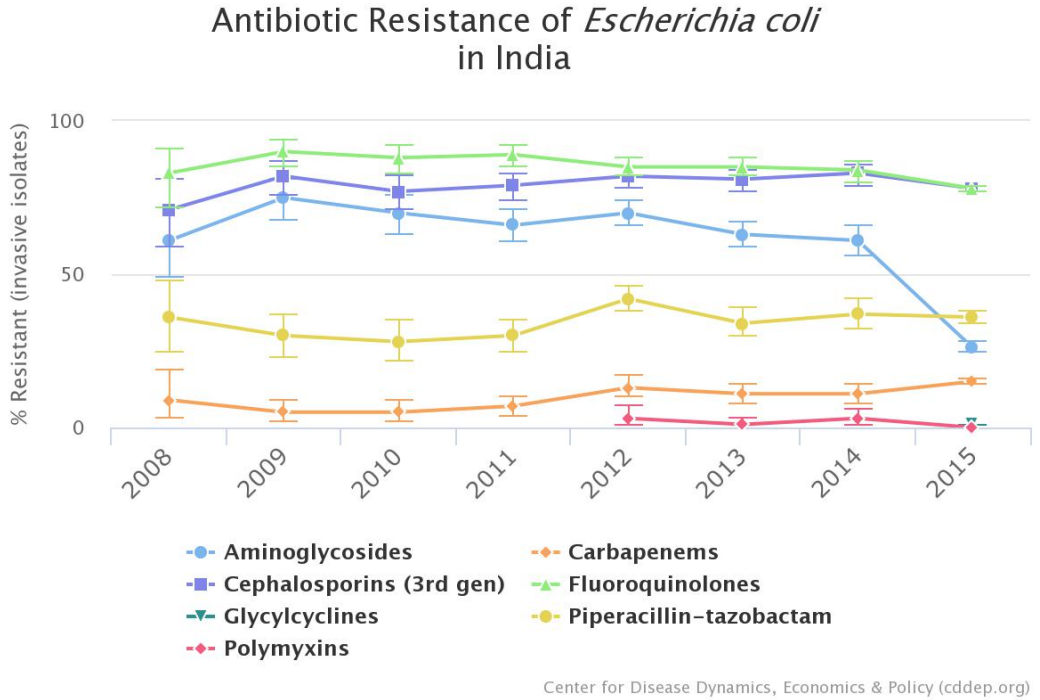
- What do you observe ?

https://resistancemap.cddep.org/About.php

Development of Antimicrobial resistance



- What do you observe ?
- Case of India

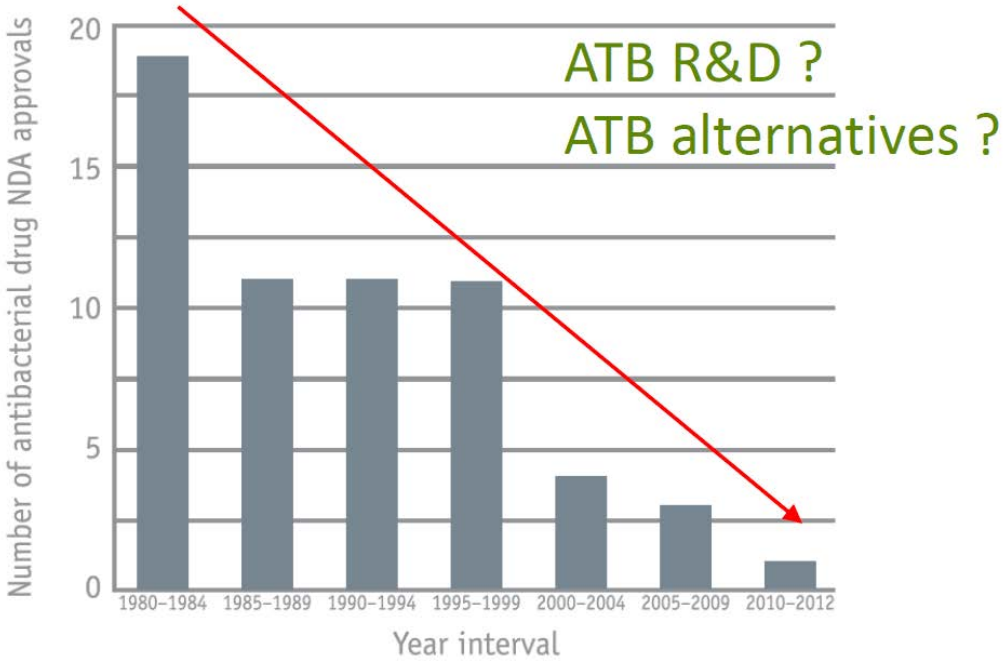


https://resistancemap.cddep.org/About.php

Development of Antimicrobial resistance

- Causes :
  - Overuse
  - Misuses
  - Use as growth promoter
  - Reduction in the number of discoveries

therapeutic deadlock ?



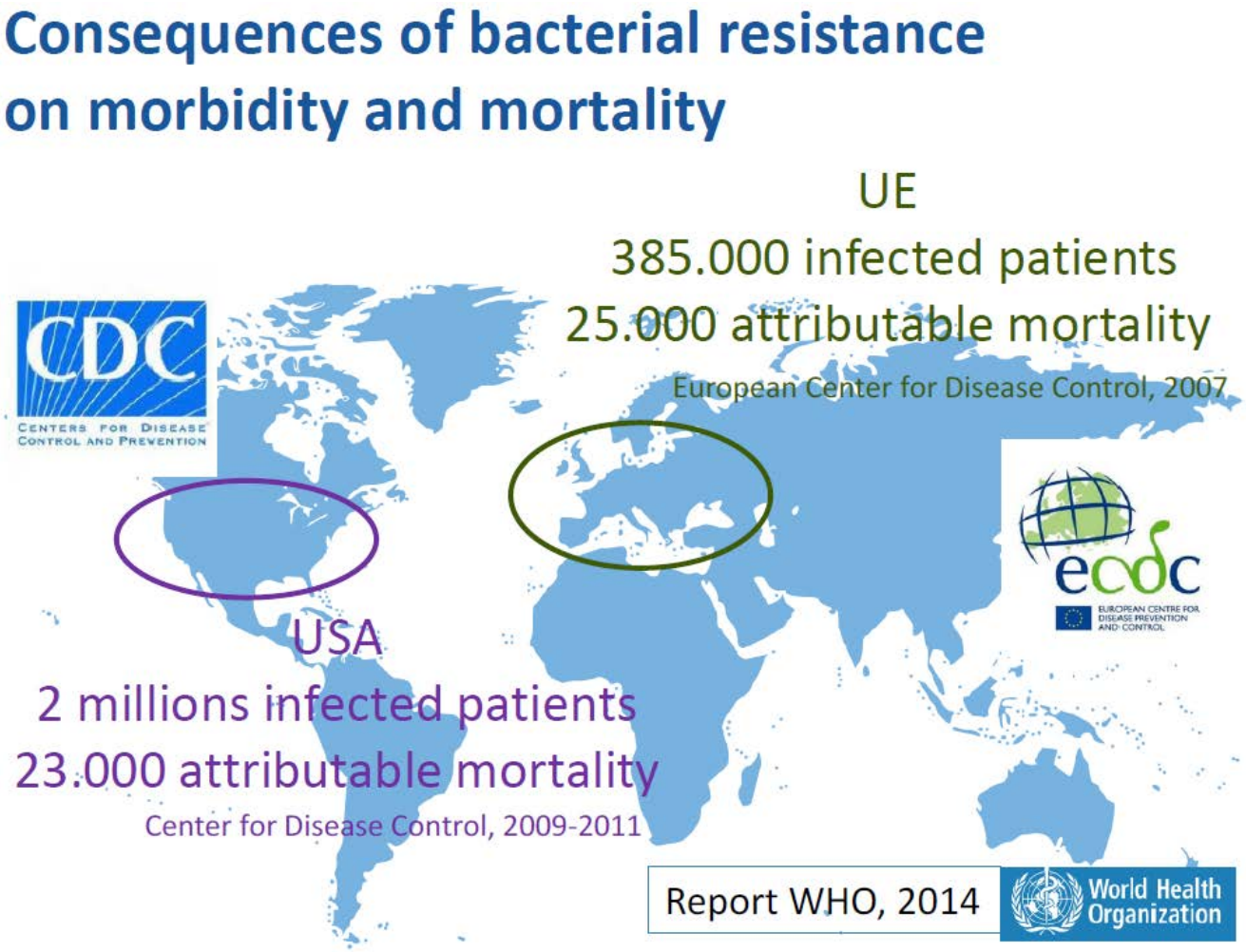
FDA Center for Drug Evaluation and Research

Development of Antimicrobial resistance

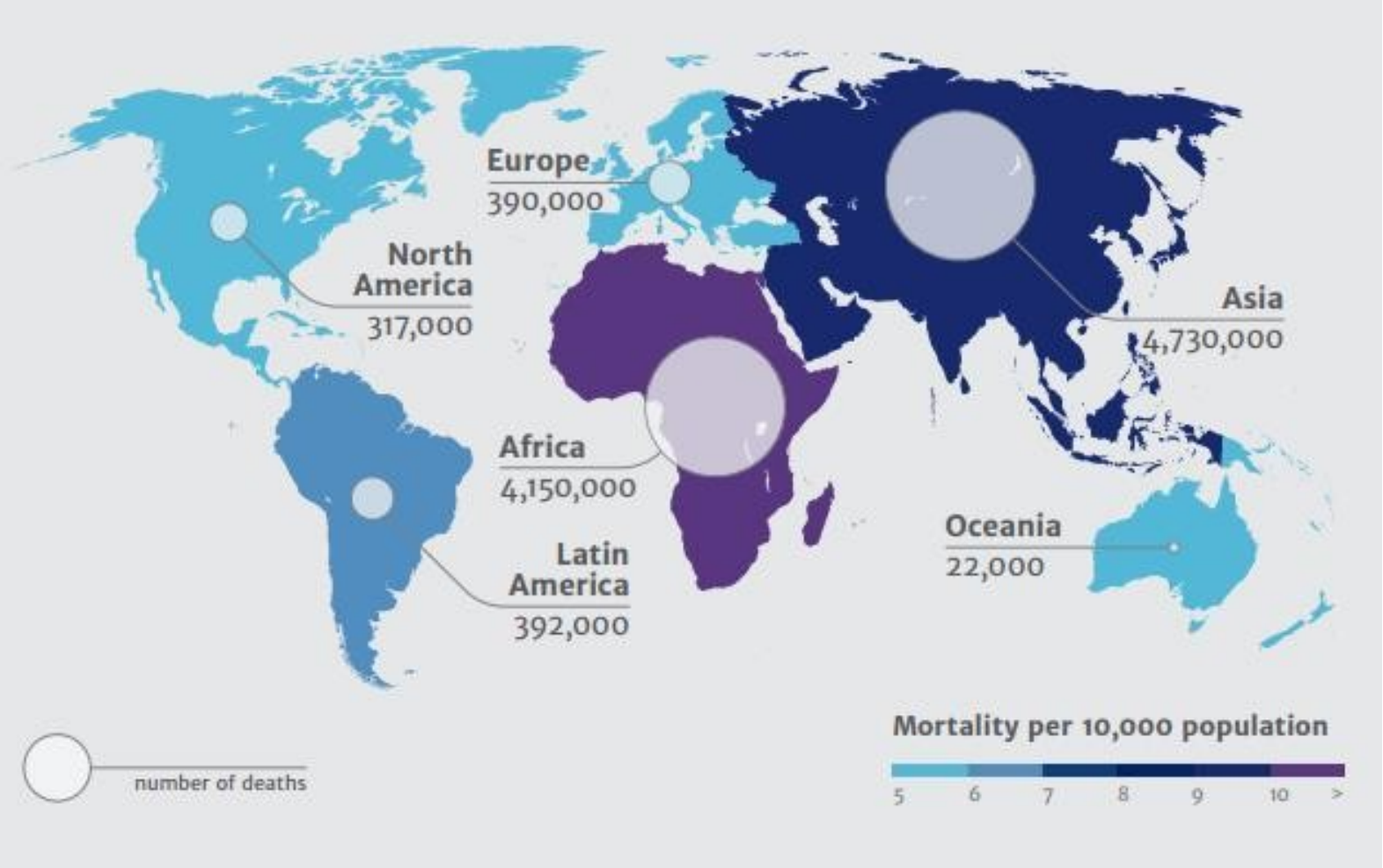
Great concern since 2000s

In June 2021, at the G7, antimicrobial resistance was described as

a **"silent pandemic“**



Development of Antimicrobial resistance



- One of the 10 greatest threats to public health (WHO)
- Significant cost: €290 million in France (2016)
- Notion of co-morbidity
- Therapeutic impasse

Deaths attributable to antimicrobial resistance every year by 2050 - Review on Antimicrobial Resistance 2014

Development of Antimicrobial resistance

Great concern since 2000s

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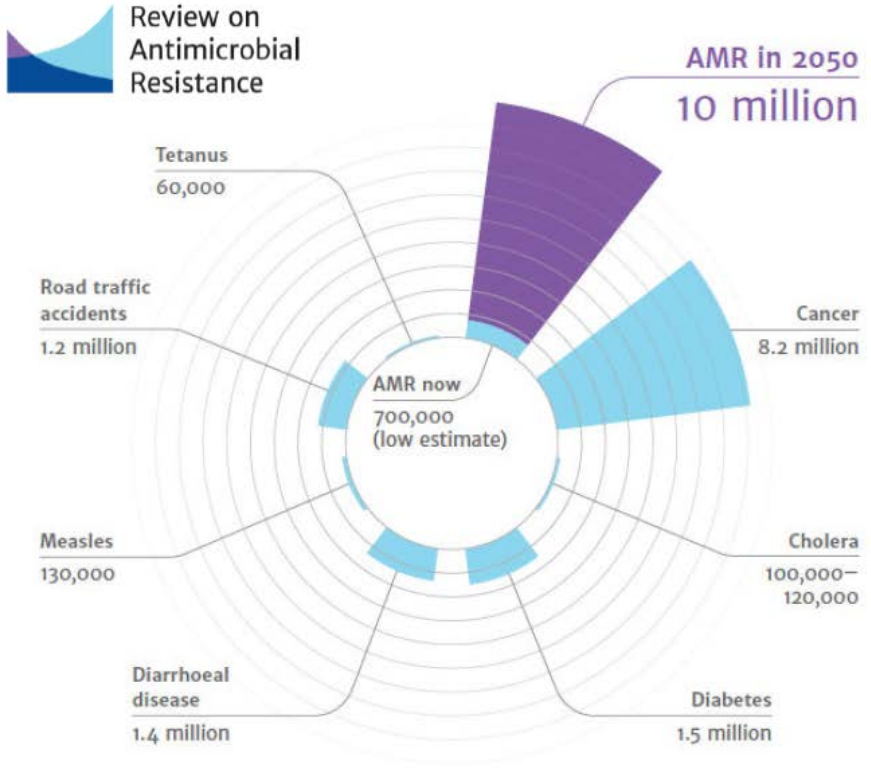
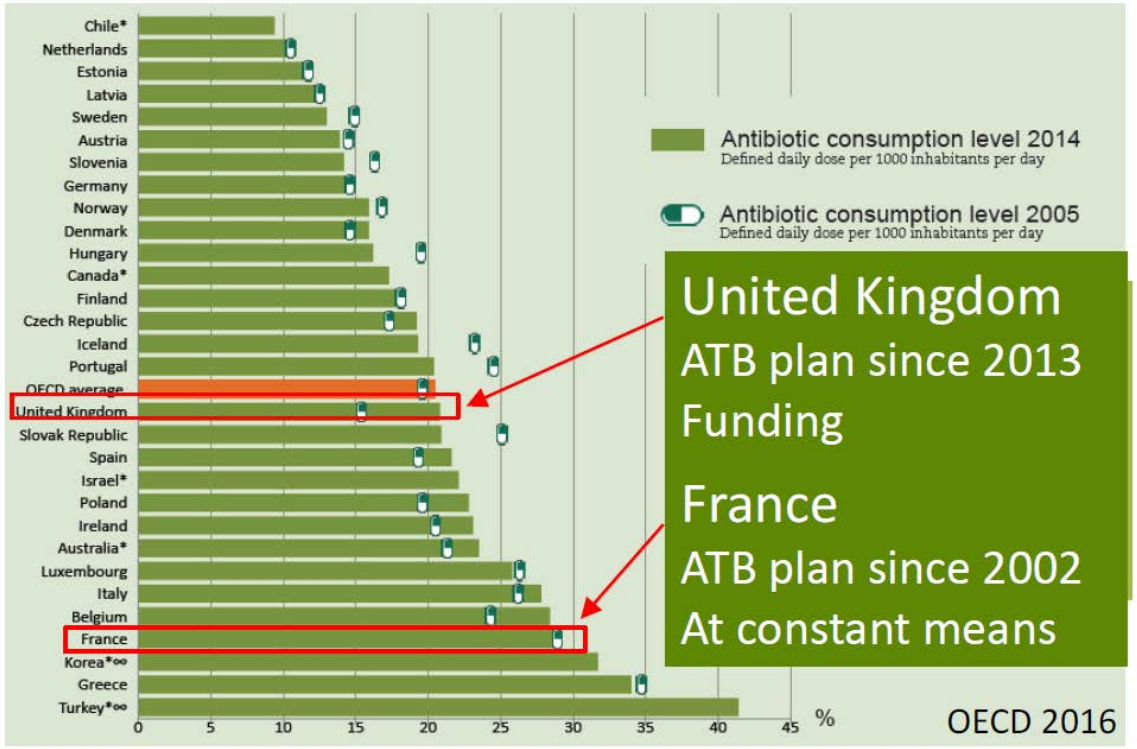
Potential consequences for the future (2015-2050)

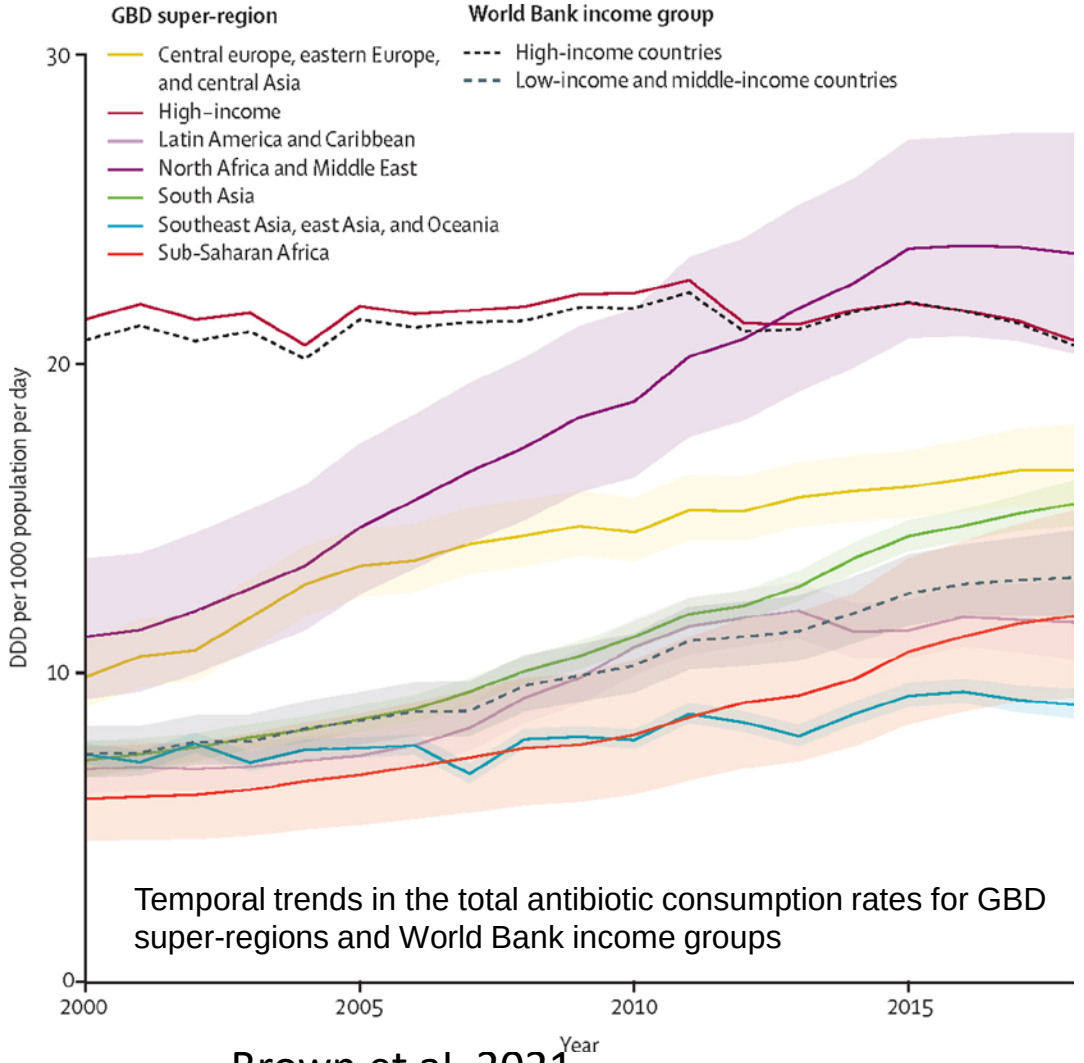


Source: The review on AMR, Jim O'Neill 2014

Evolution of consumption of ATB in humans

Antibiotics, human consumption 2005-2014, OECD





Temporal trends in the total antibiotic consumption rates for GBD super-regions and World Bank income groups

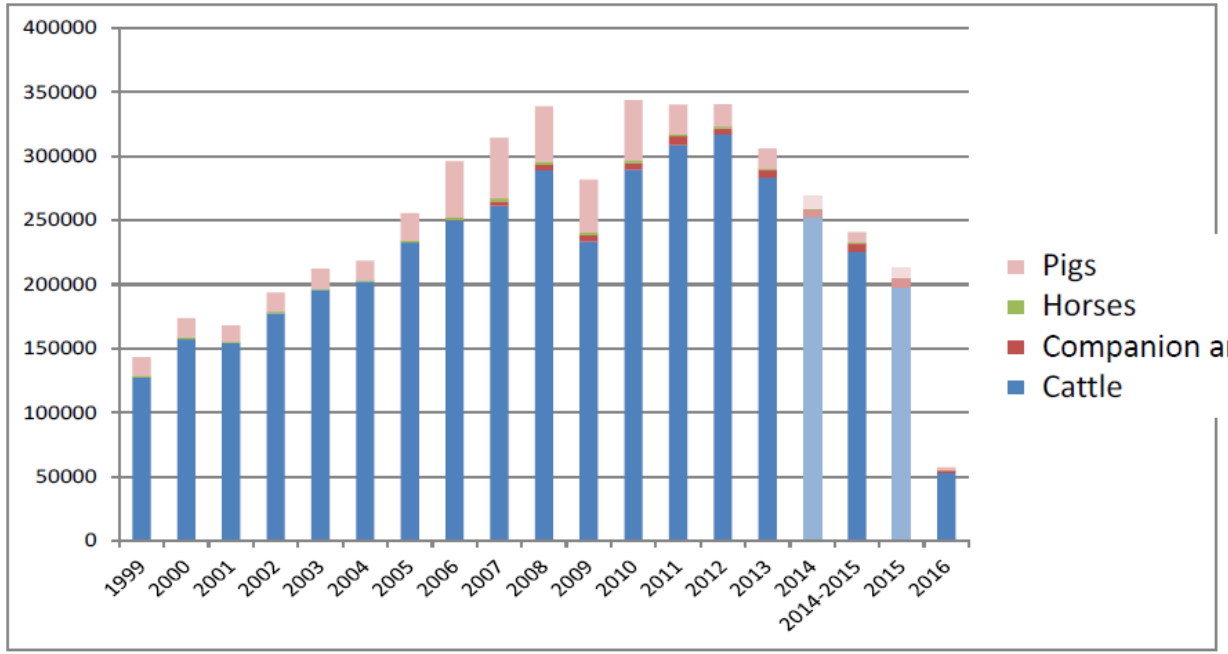
Brown et al. 2021

… In animals

- Reduce the use as growth promoters
- In 2017, In China, an emergence of Colistin resistance in humans and animals
  - More than 8 000 tons of colistin stopped in animal feed in China (April 30, 2017)
  - Still approved for treating sick animals

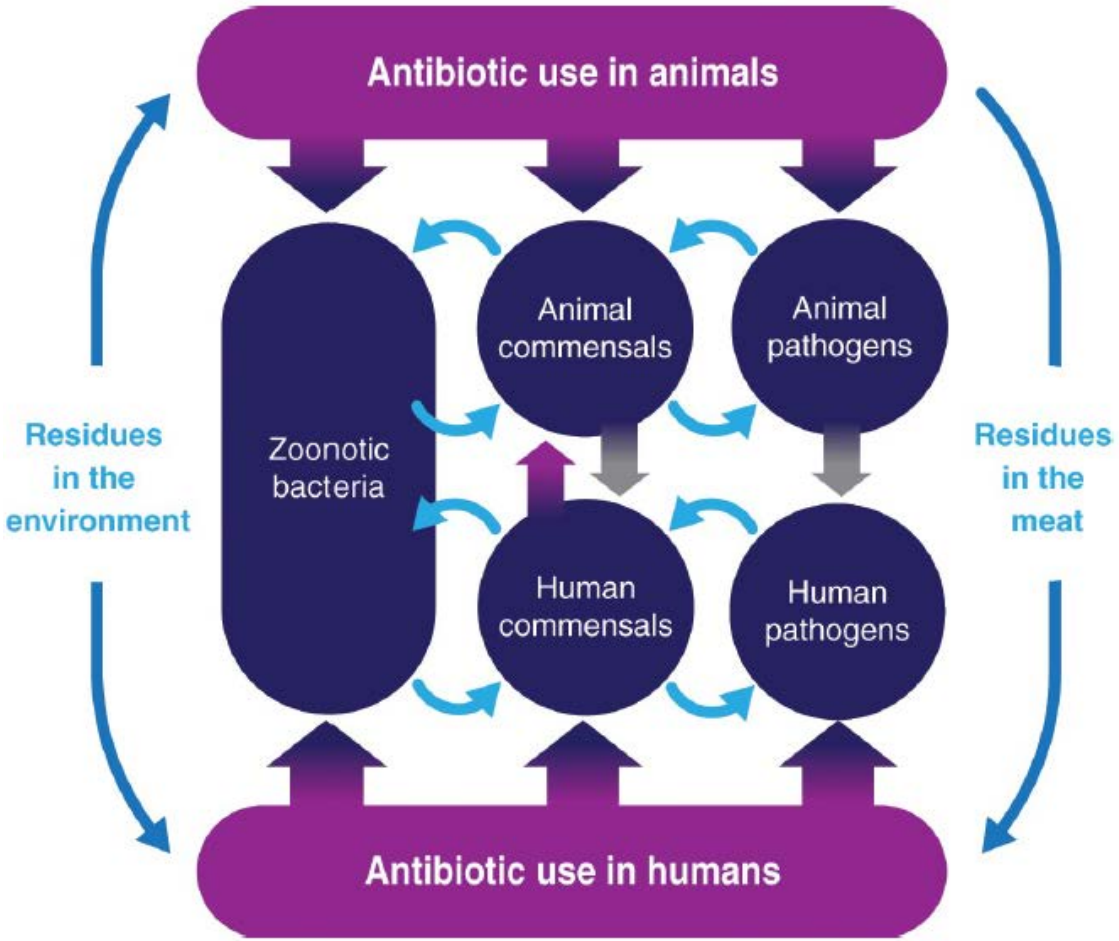
- Reduce the authorized uses

Animal weight (tons) treated with antibiotics (France)



National Agency for Veterinary Medicinal Products (France) 2017 Report

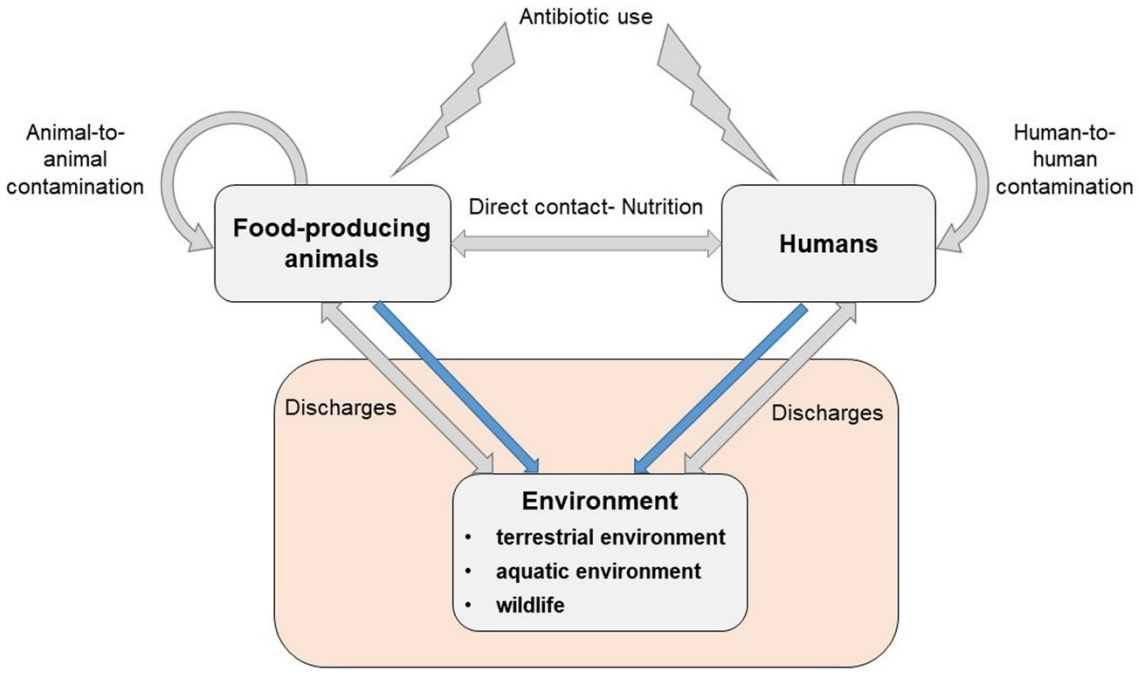
AMR in humans and animals are interconnected



Dewulf et al. 2020

- AMR in humans and animals are interconnected, that means antibiotic use both in humans and animals selects for resistance.
- In particular, antibiotic use in animals selects for resistance in animal commensals and animal pathogens.
- And later on, resistant bacteria or resistance genes can be transmitted to bacteria either from the same bacterial species or other bacterial species such as human commensals or human pathogens.
- Antibiotic use in animals can also select for resistance in zoonotic bacteria that can then be transmitted to humans and cause treatment failures.

What is the role of Environment in AMR ?



The flow of antimicrobial resistance and antibiotics across the One Health continuum of food-producing animals, humans, and the environment. Grey arrows indicate flow of resistance genes and resistant bacteria; blue arrows indicate flow of antibiotic residues.

Haenni et al. *Environ Int 2022*

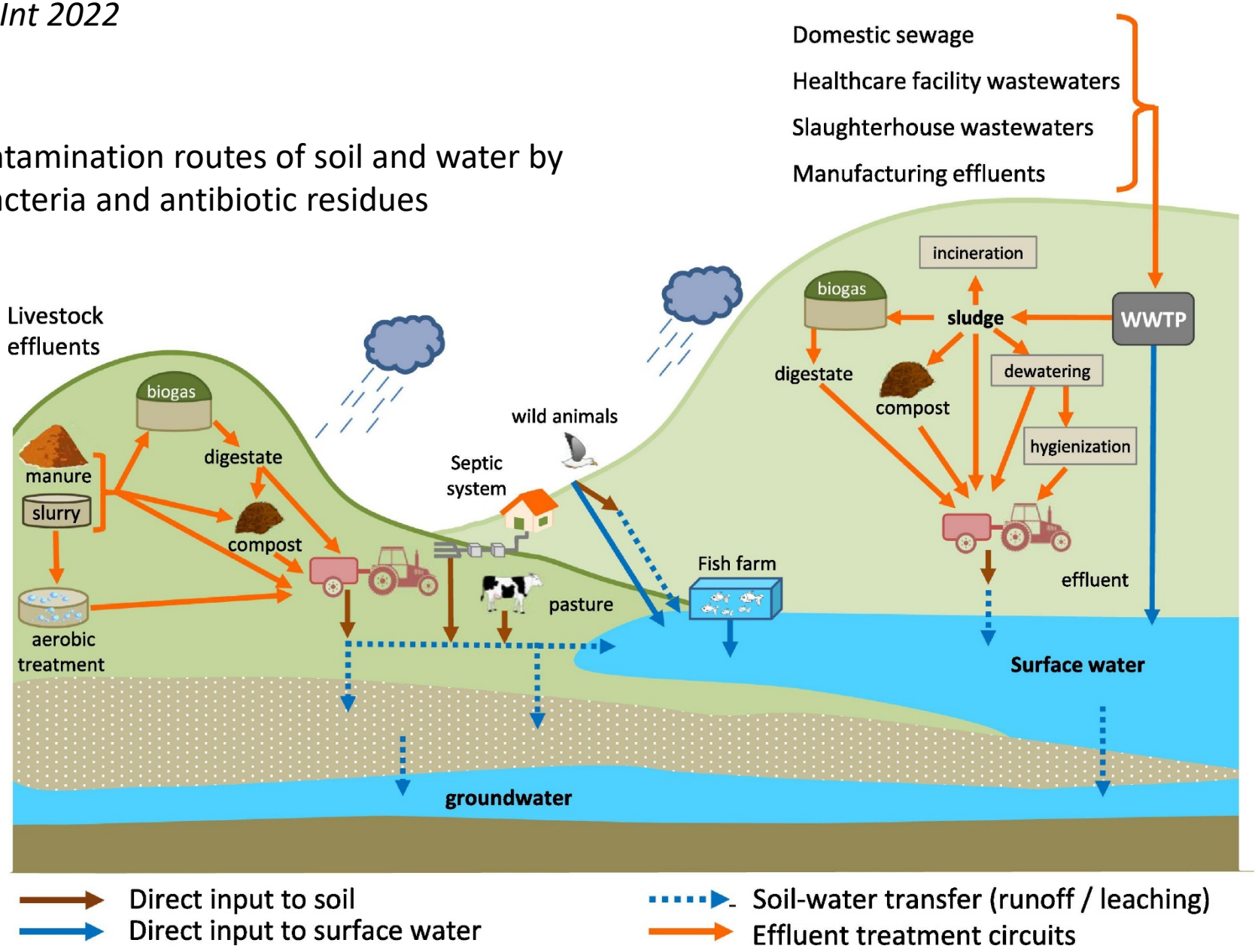
- Under antibiotic pressure
- AMR development
- ATB and AMR release in environnement

In environment, are concentrations levels of ATB and bacteria sufficient to participate to the AMR ?

How ATB enter the environment ?

Haenni et al. *Environ Int 2022*

Main sources and contamination routes of soil and water by antibiotic-resistant bacteria and antibiotic residues



Sources and pathways in the environment

Pharmaceutical industries

Antibiotics

effluent

Waste water
treatment Plant

Sources and pathways in the environment

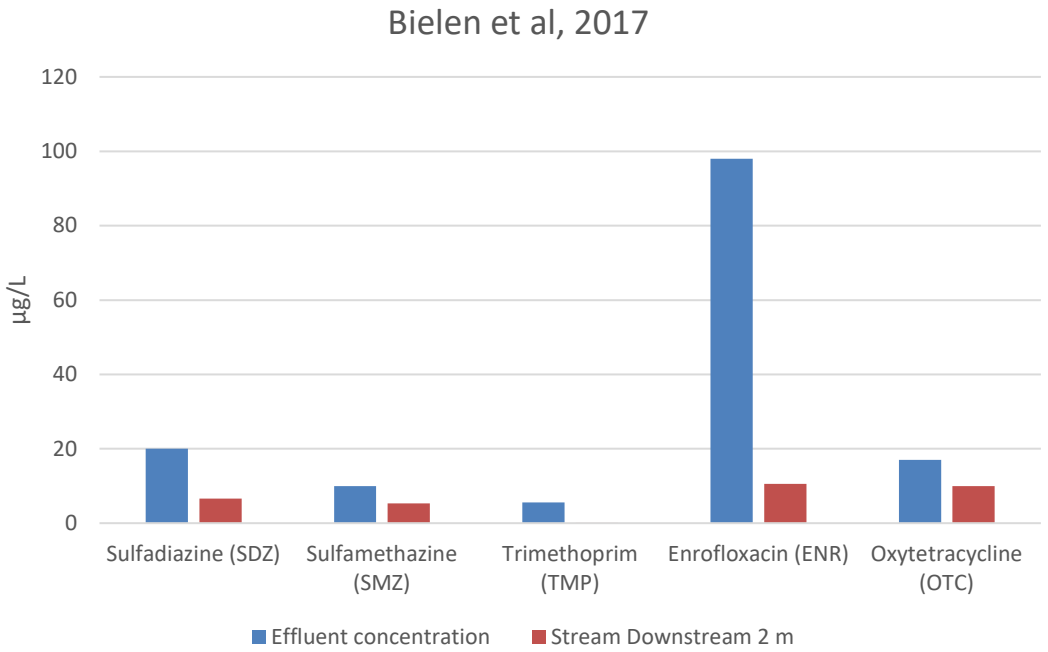
Antibiotics

Pharmaceutical industries

Effluent without Treatment

effluent

Waste water treatment Plant



Concentration of five antibiotics, expressed in µg/L, in effluents of Company 2 and in the recipient stream.

Sources and pathways in the environment

Pharmaceutical industries

Antibiotics

Human medicine

Town medicine

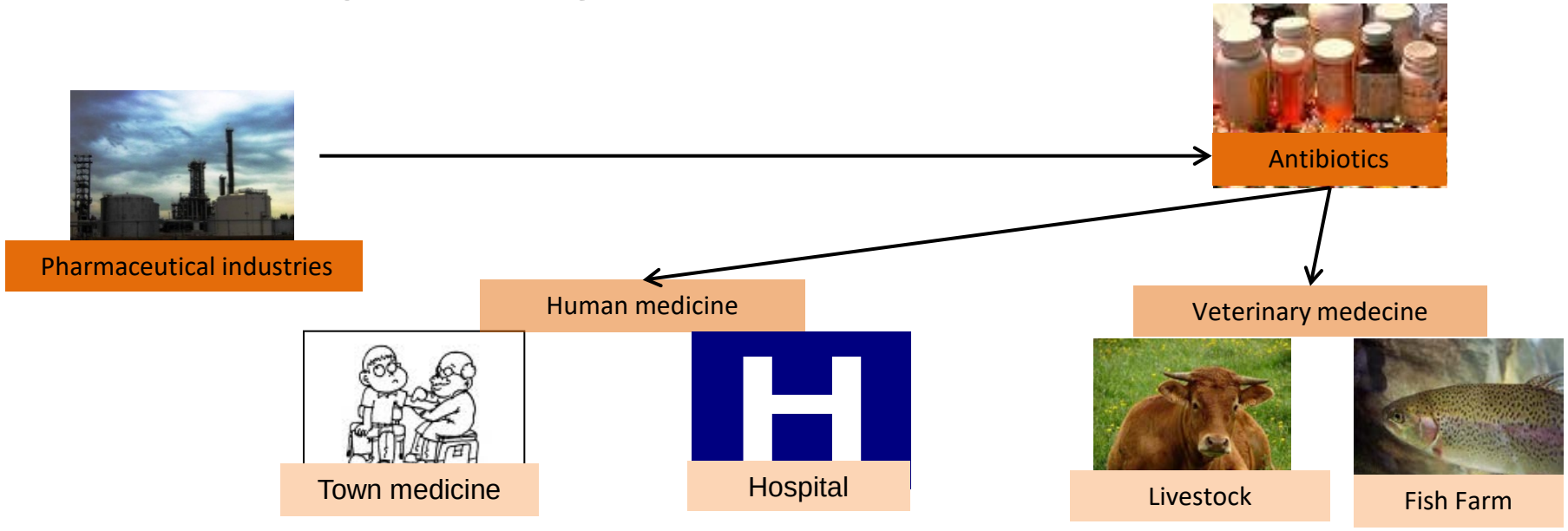
Hospital

Veterinary medecine

Livestock

Fish Farm

Sources and pathways in the environment



Quantities consumed per year in France

Antibiotique	Tonnes/an
Pénicillines	**500**
Streptogramines	50
Céphalosporines (C3G et C4G)	35
Fluoroquinolones	25

Antibiotique	Tonnes/an
Tétracycline	180
Sulfamides	90
Pénicillines	71
Aminosides	53

Ansm 2020 ; pour la médecine vétérinaire : Anses/ANMV 2020

Sources and pathways in the environment

Pharmaceutical industries

Antibiotics

Human medicine

Town medicine

Hospital

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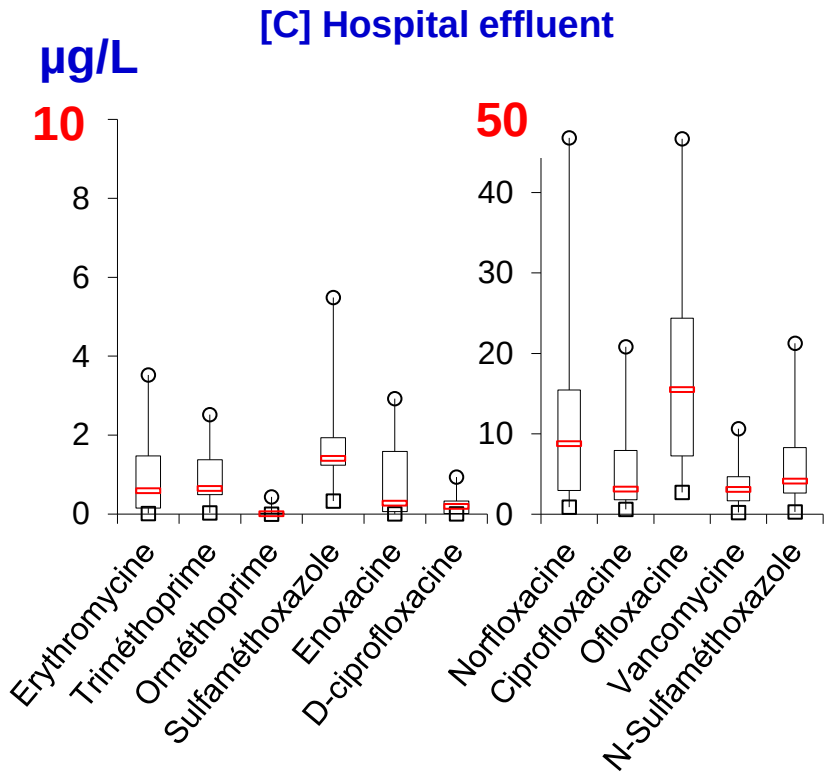
⇨ Excreted as unchanged or metabolized form

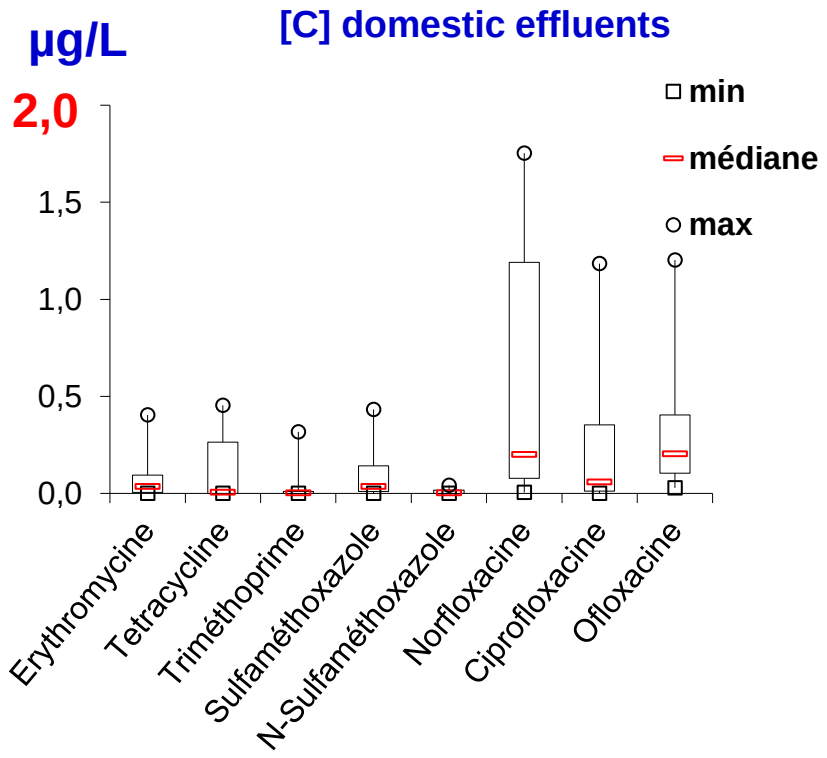
Human excretion rates

		Taux d'excrétion %	
		Inchangé	Métabolisé
Macrolides	Tylosine	28 - 76 [a]	
	Erythromycine	> 60 [b]	
Tétracyclines	Chlorophenicole	5 - 10 [a]	
	Tétracycline	80 - 90 [a]	
	Chlorotétracycline	< 70 [a]	
	Oxytétracycline	< 80 [a]	
β-lactamines	Amoxiciline	80 - 90 [a]	10 - 20 [a]
	Ampiciline	30 - 60 [a]	20 - 30 [a]
	Penicilline G	50 - 70 [a]	30 - 70 [a]
Sulfodinamides	Sulfaméthoxazole	15 [a]	
	Sulphaméthoxine	~ 15 [a]	
Diaminopyrimidines	Triméthoprime	50 [b]	
Quinolones et	Acide nalidixique	20 [b]	
Fluoroquinolone	Fluméquine	10 [b]	
	Norfloxacine	70 [b]	
	Ofloxacine	80 [b]	
	Ciprofloxacine	75 - 85 [b]	
Imidazoles	Ornidazole	5 [b]	
Glycopeptides	Vancomycine	**90** [b]	
Aminoglycosides	Streptomycine	< 66 [a]	

a : (Jjemba, 2002), b : (Tamtam, 2008)

Human medecine

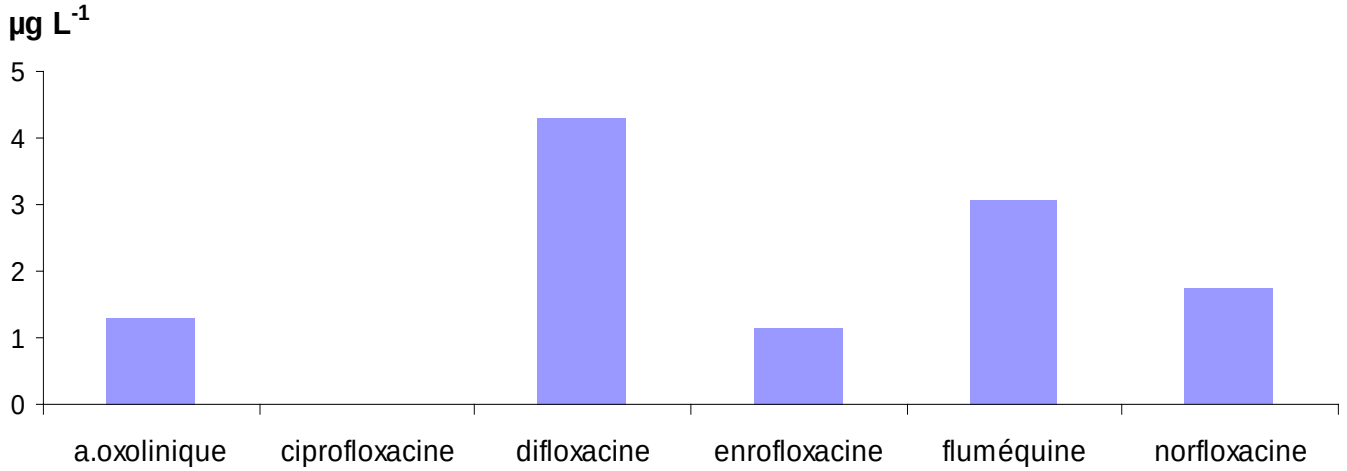




Dinh et al, 2017

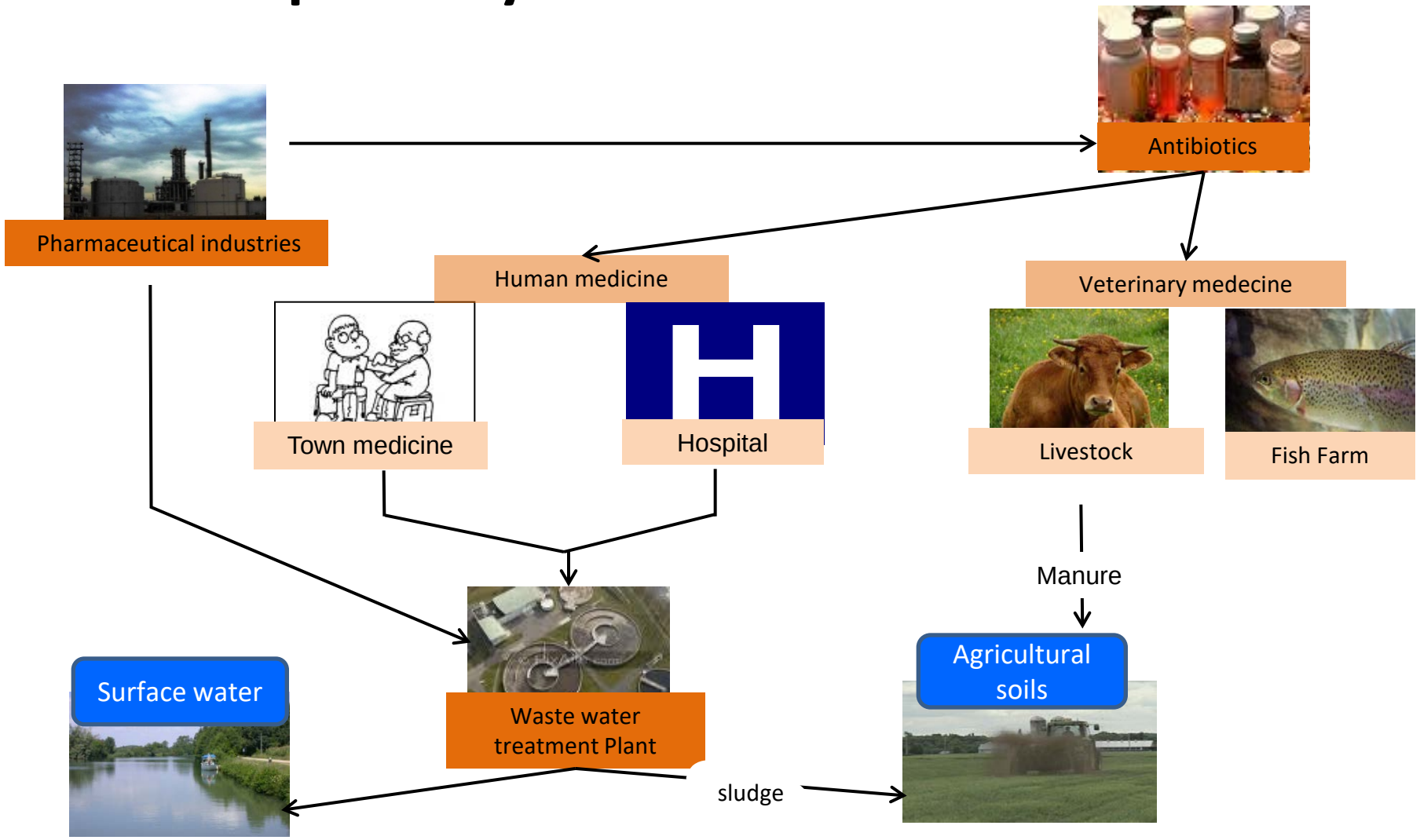
- [ATB] in hospital effluent > Domestic effluent
- Vancomycine only found in hospital effluent

Veterinary medecine



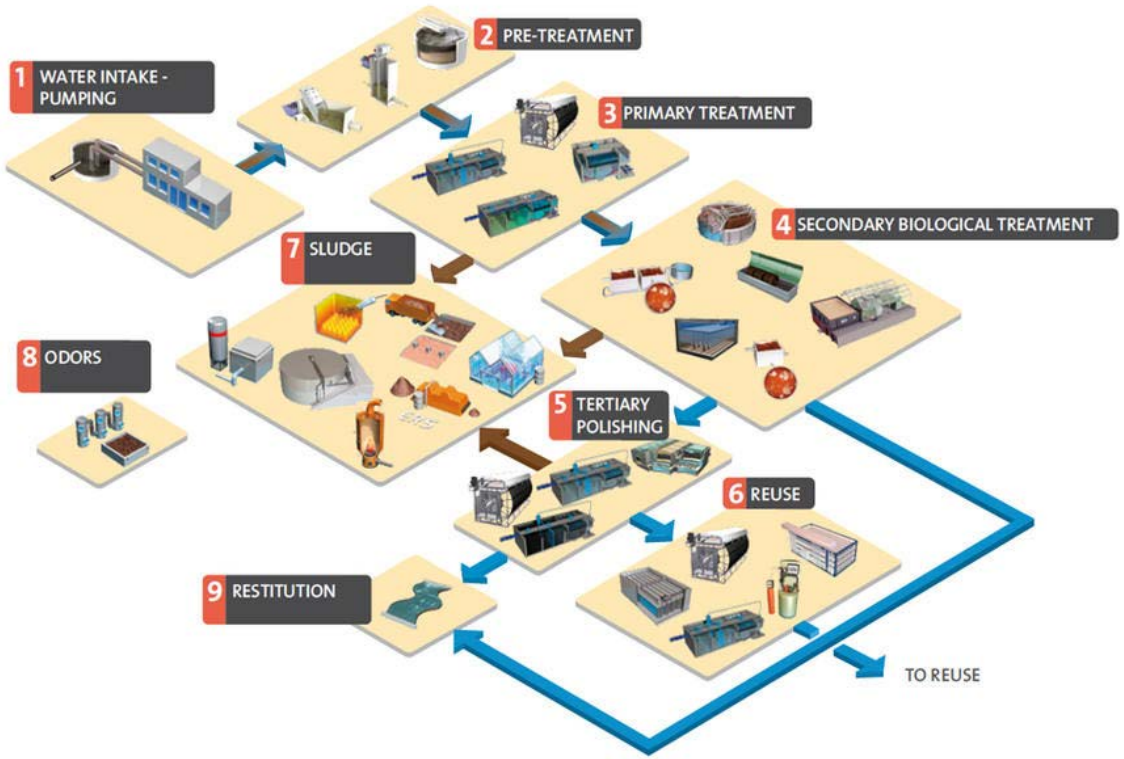
[ATB] in manure in Blaise Catchment, France (Tamtam, 2008)

Sources and pathways in the environment



Tamtam, 2008)

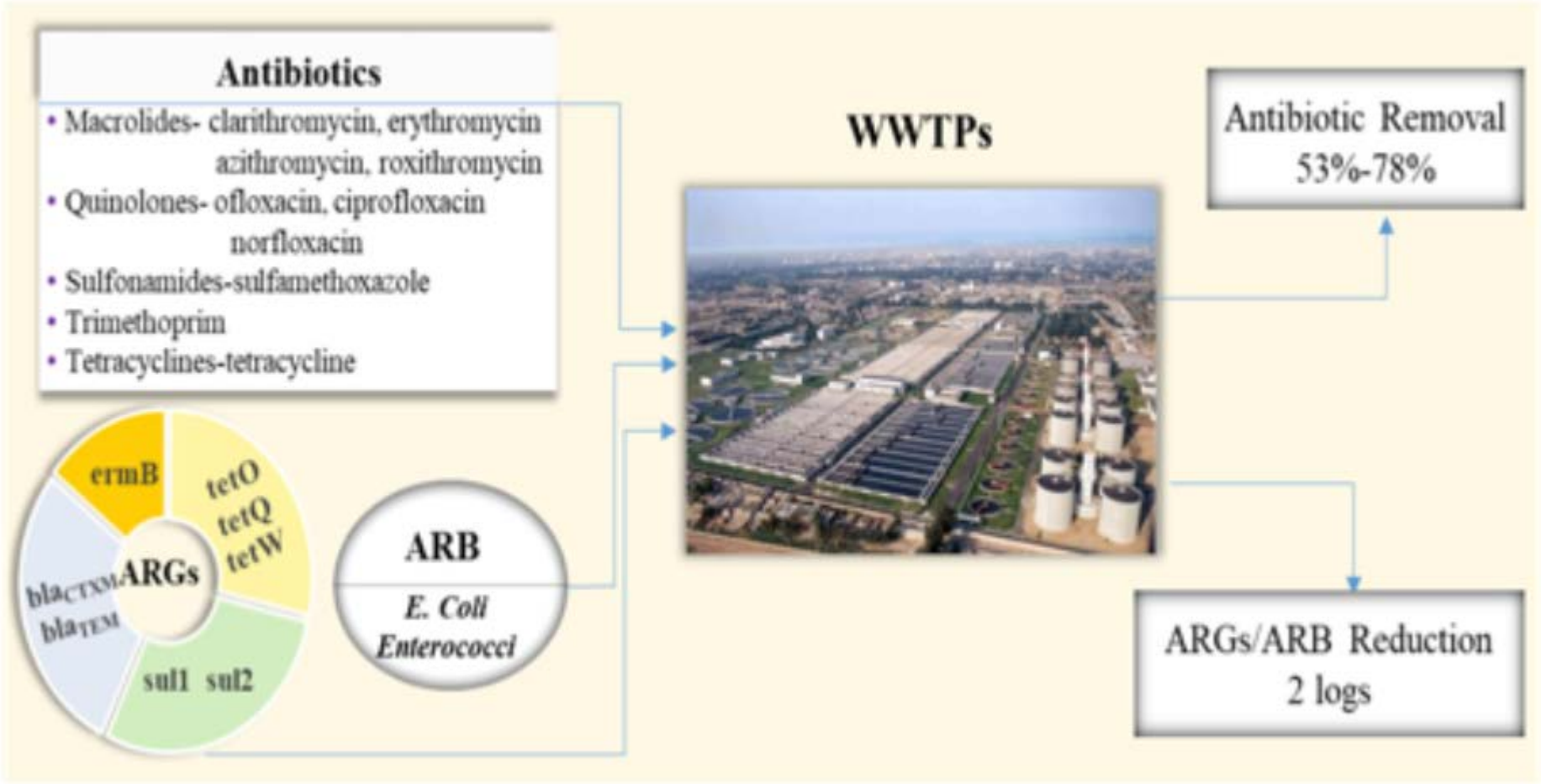
Waste water treatment plant



Veolia

1. **Primary treatment:** separating suspended solids (SS) from wastewater by flocculation / coagulation / settling and flotation processes –
2. **Secondary treatment**: advanced biological treatment methods, leveraging the ability of certain bacteria, to eliminate dissolved pollutants contained in wastewater – such as carbon, nitrogen and phosphorus pollutants.
3. **Tertiary treatment and reuse**: this final stage looks to remove any remaining dissolved solids from purified water and disinfected wastewater so that the treated water can be reused for other purposes (chlorination, ozonation, UV treatment)
4. **Quaternary treatment** : elimination of organic micropollutants by adsorption on activated carbon or ozonation
5. **Sludge treatment**: the pollutants eliminated during treatment operations become sludge, and recycling

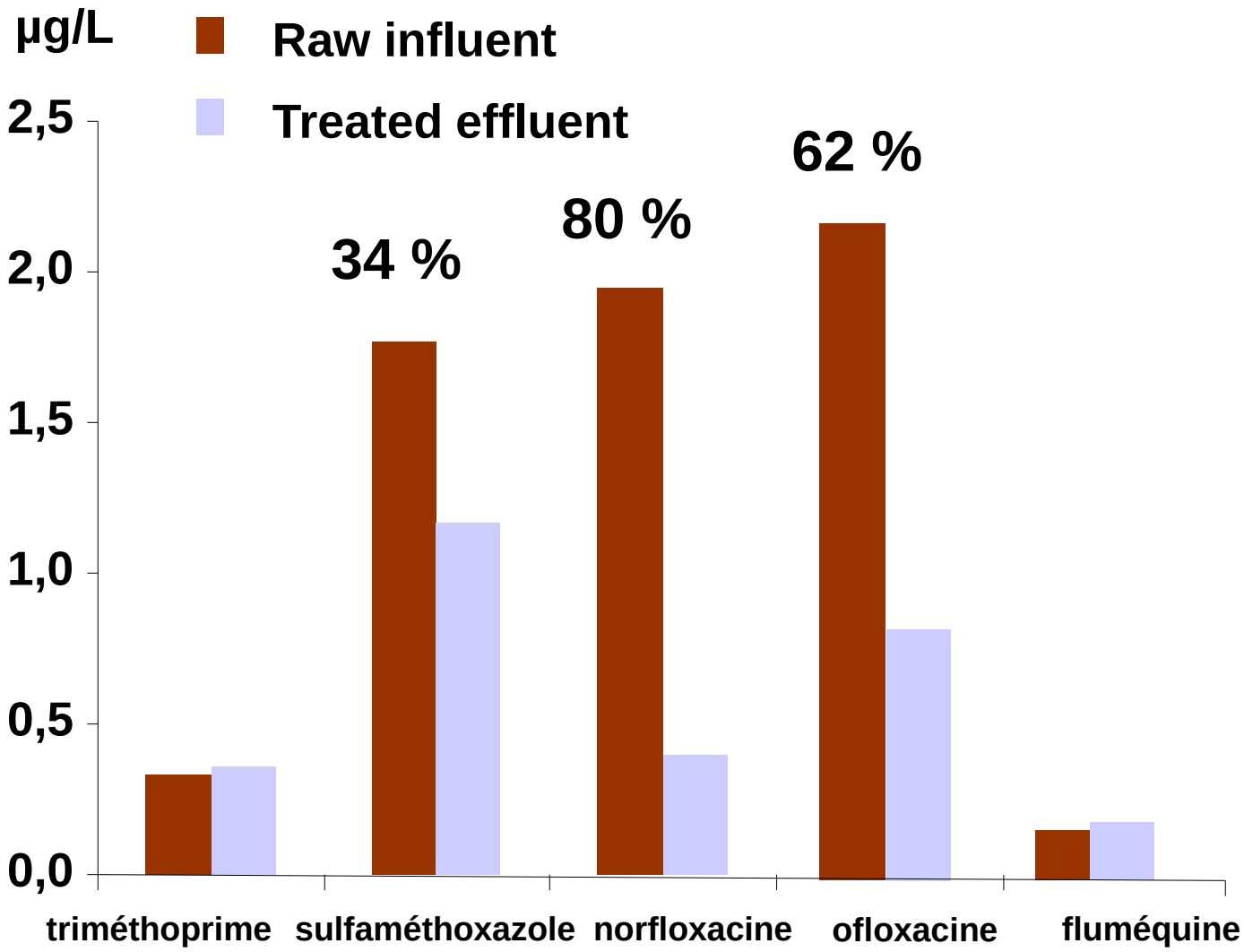
Removal of ATB (and bacteria) by WWTP



Source : Wang *et al.*, 2020

Removal of ATB (and bacteria) by WWTP

- In France,
- ATB (and bacteria) removal depend on technology used !
  - Membranes
  - Biofiltrations
  - Nanofiltrations
- Impact of ATB on WWTP efficiency (biological treatment)

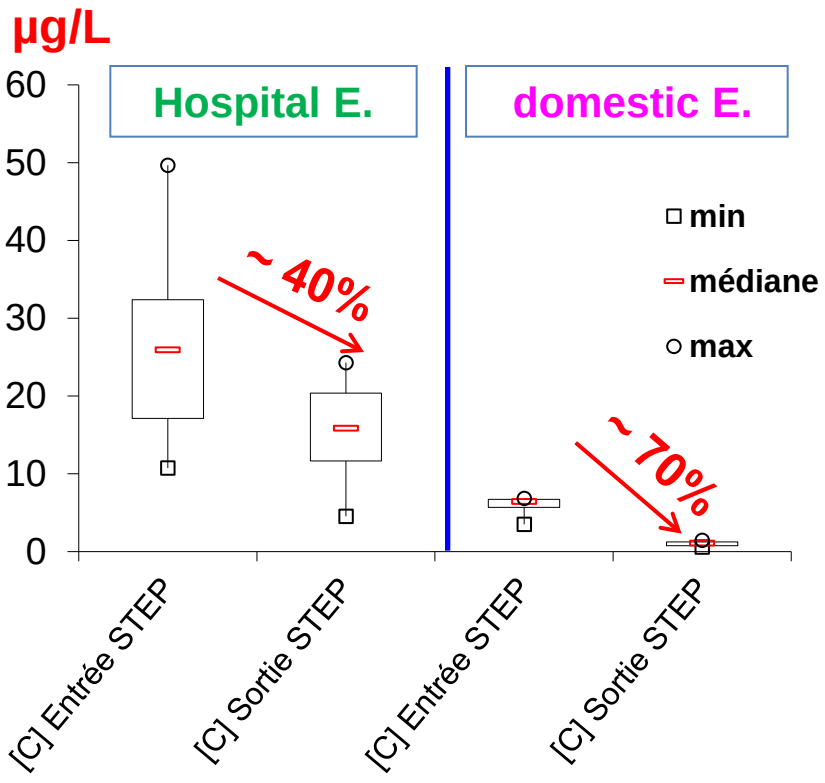


Colloque PIREN Seine, février 2008

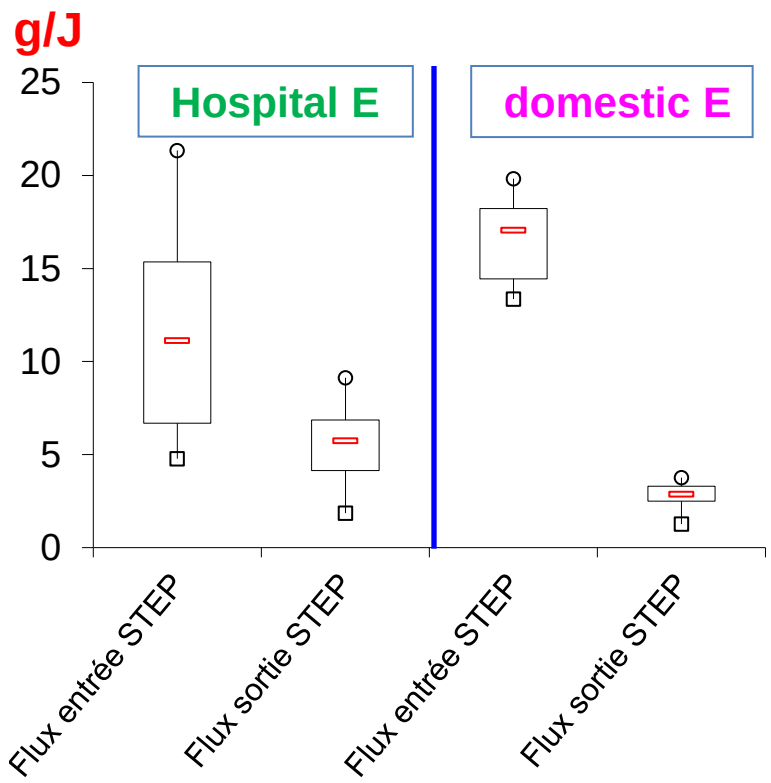
Hospital vs domestic WWTP

Hospital WWTP
1 700 inhab
+ Hospital (240 beds)

Sum of ATB concentrations in entrance and exit



Daily fluxes

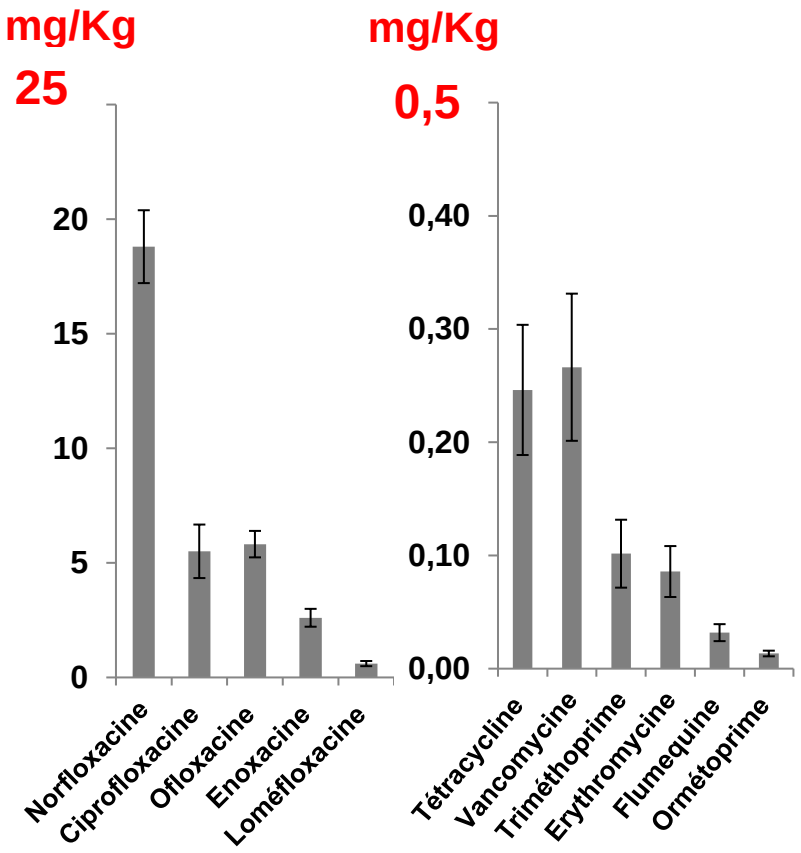


Domestic WWTP
City 15 000 inhab

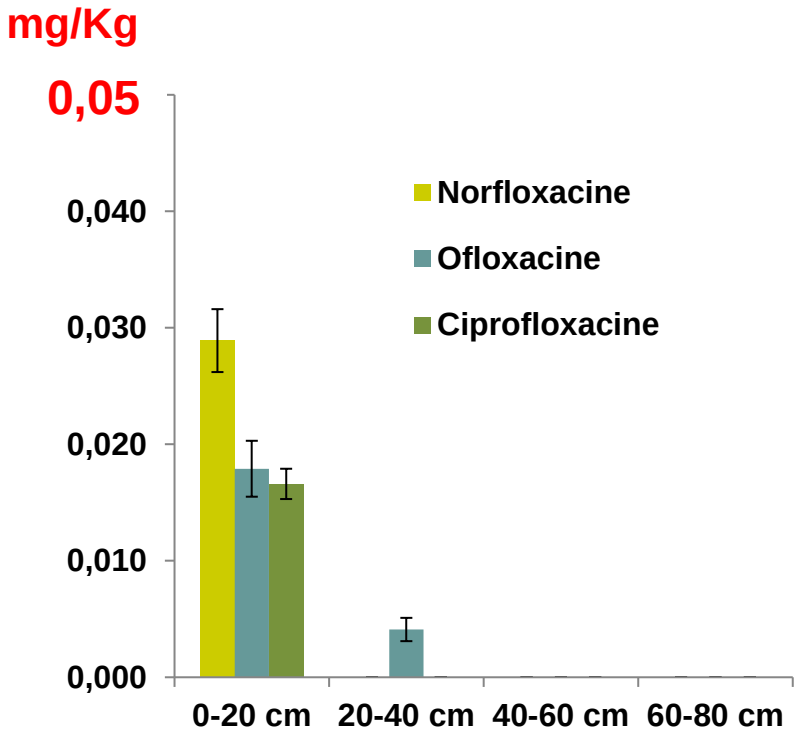
Dinh et al, 2017

Sludge and soil contaminations

ATB content in sludge (Hospital WWTP)



ATB contents in soil after sludge spreading



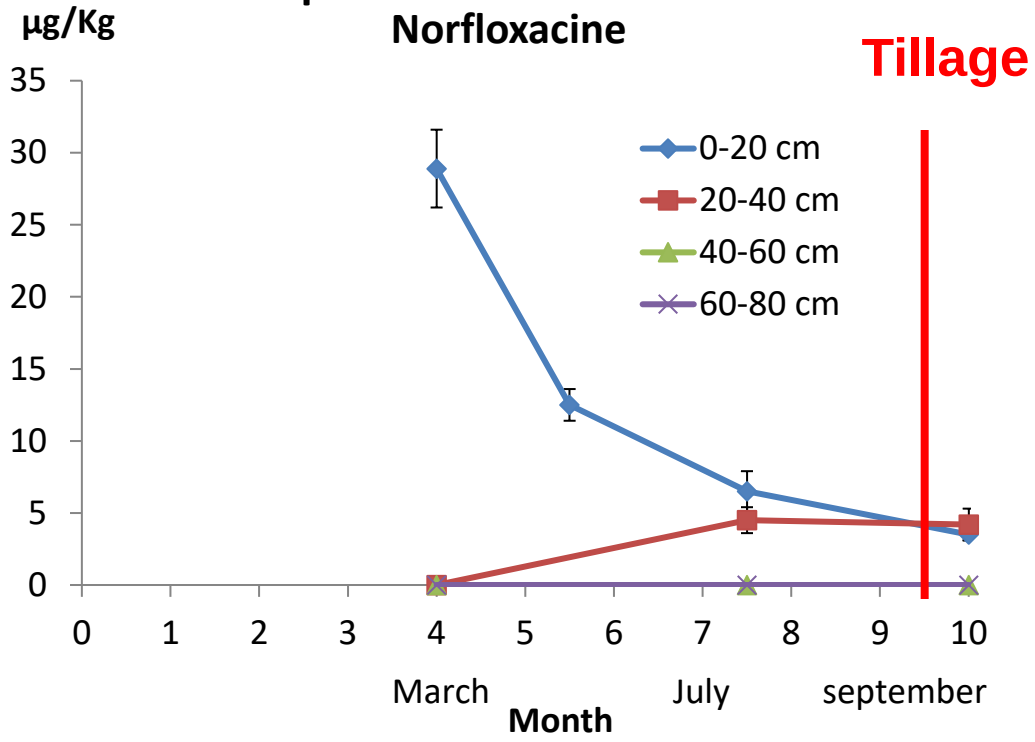
- **Fluoroquinolones : few mg/Kg**
- **Others molecules : < 0,5 mg/Kg**

3 fluoroquinolones : < 0,05 mg/Kg

Dinh et al, 2017

Fate of ATB contents in soils

- Case of fluoroquinolones



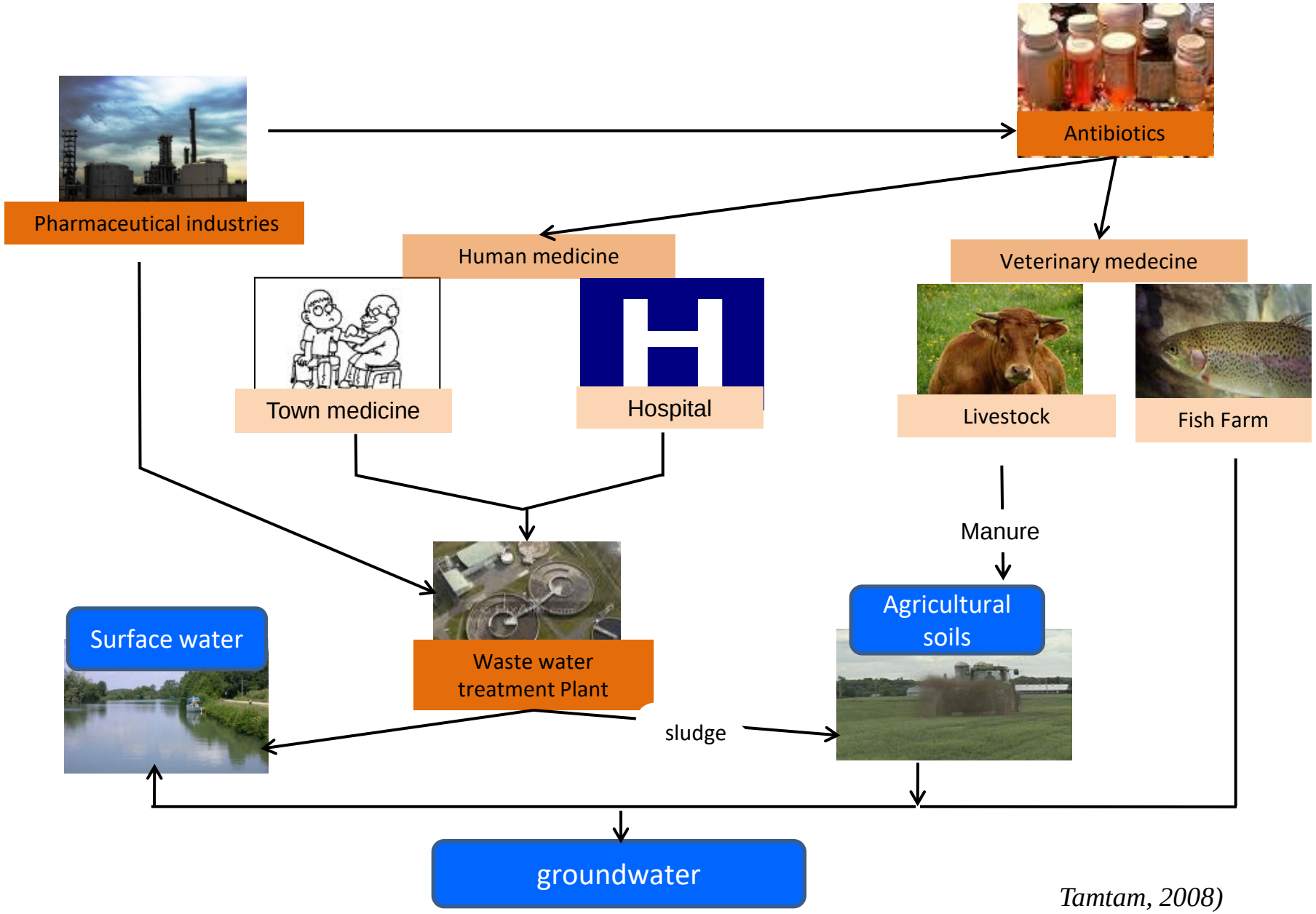
- Source : only sludge
- No ATB in depth
- No impact of tillage

⇨ Degradation of compounds

Importance of Physico-chemical properties of molecules (persistance, Half-life, solubility, adsorption…)

Reminder : Origin of ATB : Bacteria and fungy in soils !!!

Sources and pathways in the environment



Tamtam, 2008)

Fish farm

Permanent treatment (antibiotics in feed) -> preventive treatment

Occasional treatment of a pond

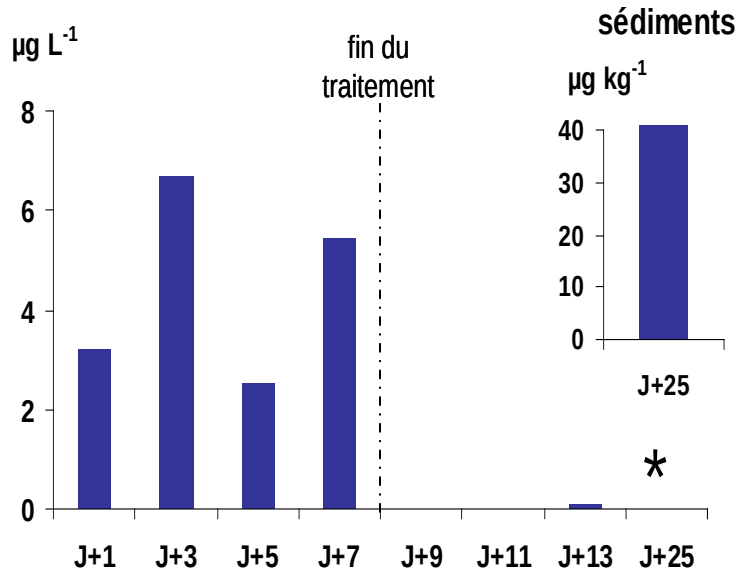
Environmental Impact of Acide oxolic and Flumequine used in fish farm

Direct introduction of ATB in water

- from undigested feed (dissolved form)
- fractions excreted by fish (form associated with solid material: feed, faeces)

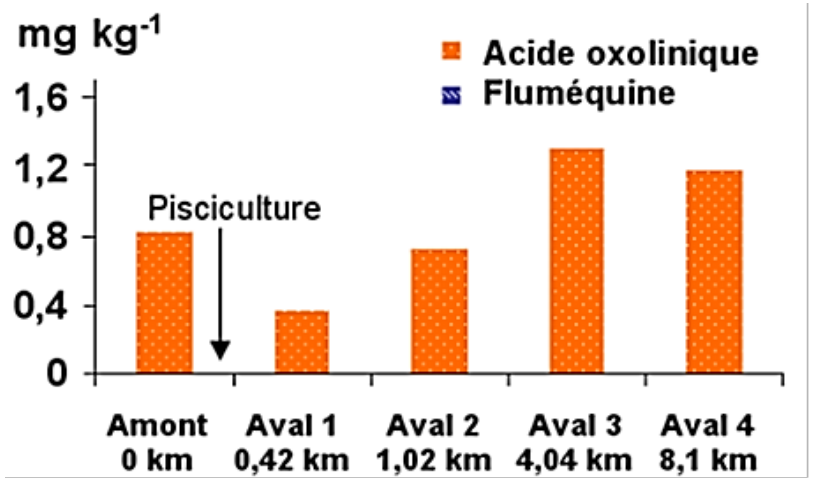
Cravedi et al [1987], 90% of the administered treatment is not metabolised by the fish.

During an occasional Treatment by Flumequine



Concentrations of Flumequine in river

No treatment period



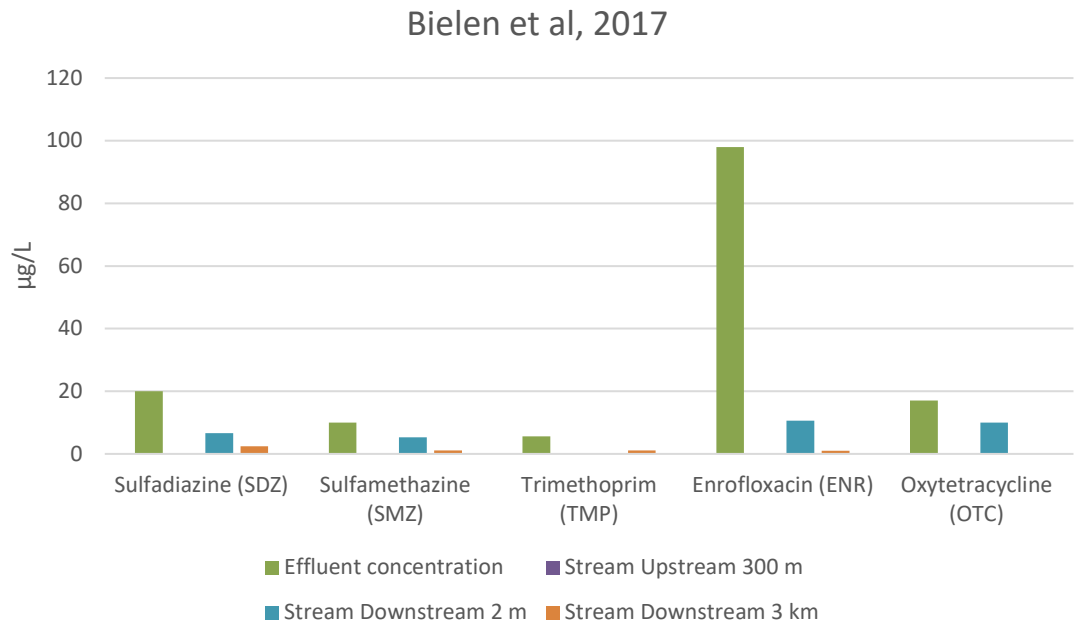
Concentrations of Ac Oxolinic in sediment river

No ATB in water, only Ac Oxolinic in sediment

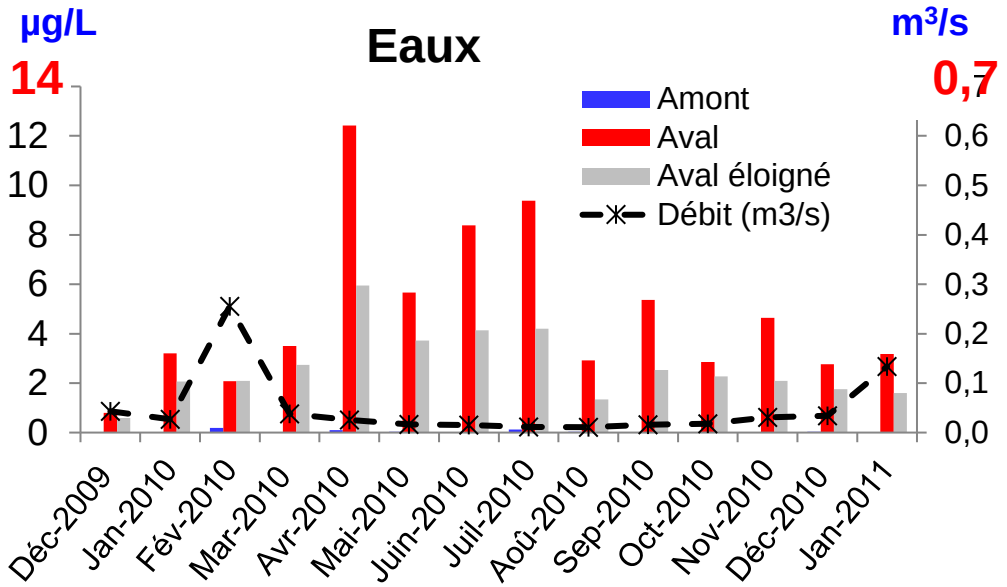
Tamtam, 2008

Surface water contamination

- By pharmaceutical industries



- By WWTP (hospital)



Dinh et al, 2017

Water and Sediment contamination

Impact of WWTP effluent

Upstream : low

Dowstream :
- water : ~ 4 µg/L
- Sediments: ~ 2 mg/Kg

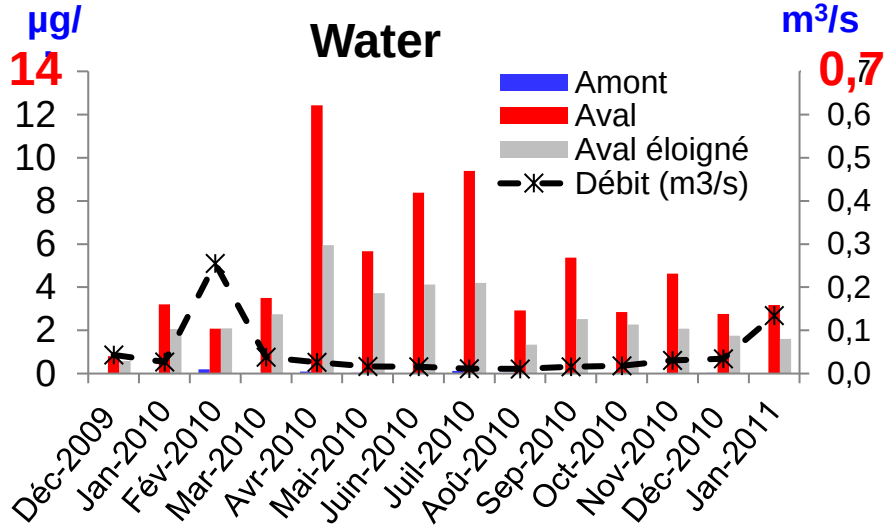
decrease in distance from the discharge

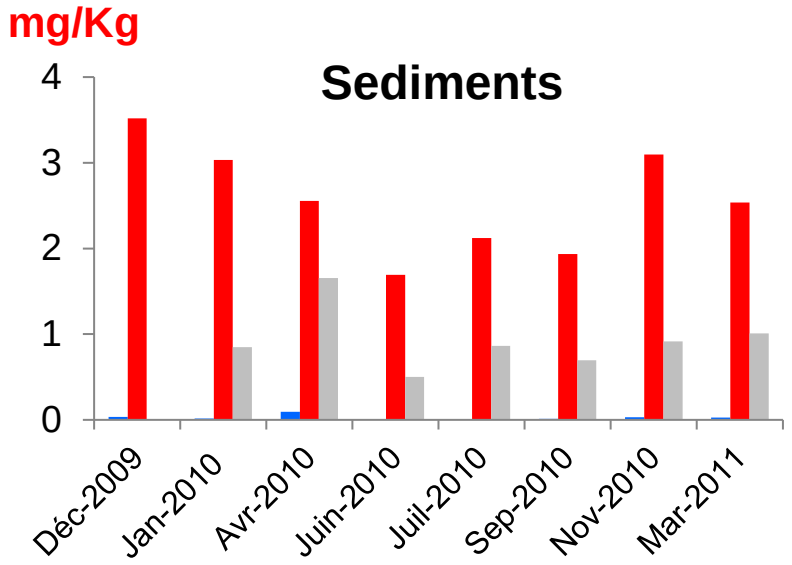
In Water: fluoroquinolones et sulfonamide (downtream)

Far Downstream: sulfonamides mainly

Sediments : ~ 90% fluoroquinolones

Dissipation of fluoroquinolones in water ~ adsorbed in sediment

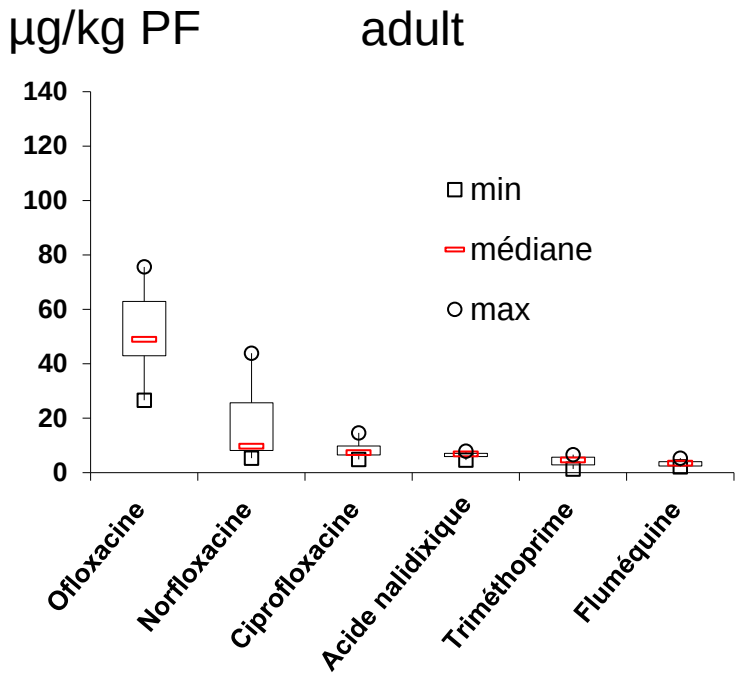


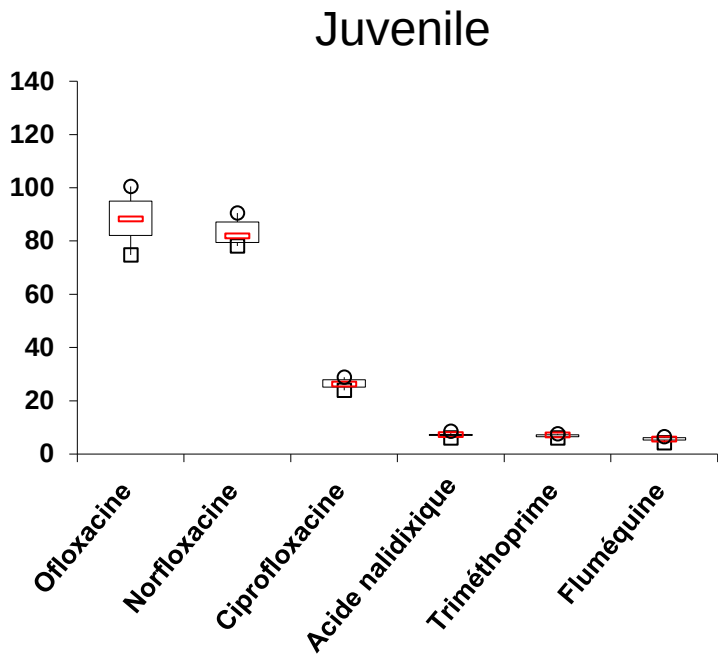


Dinh et al, 2017

Impact on the ecosystem

- Bioaccumulation of ATB in Invertebrate species (Gammarus Pulex)

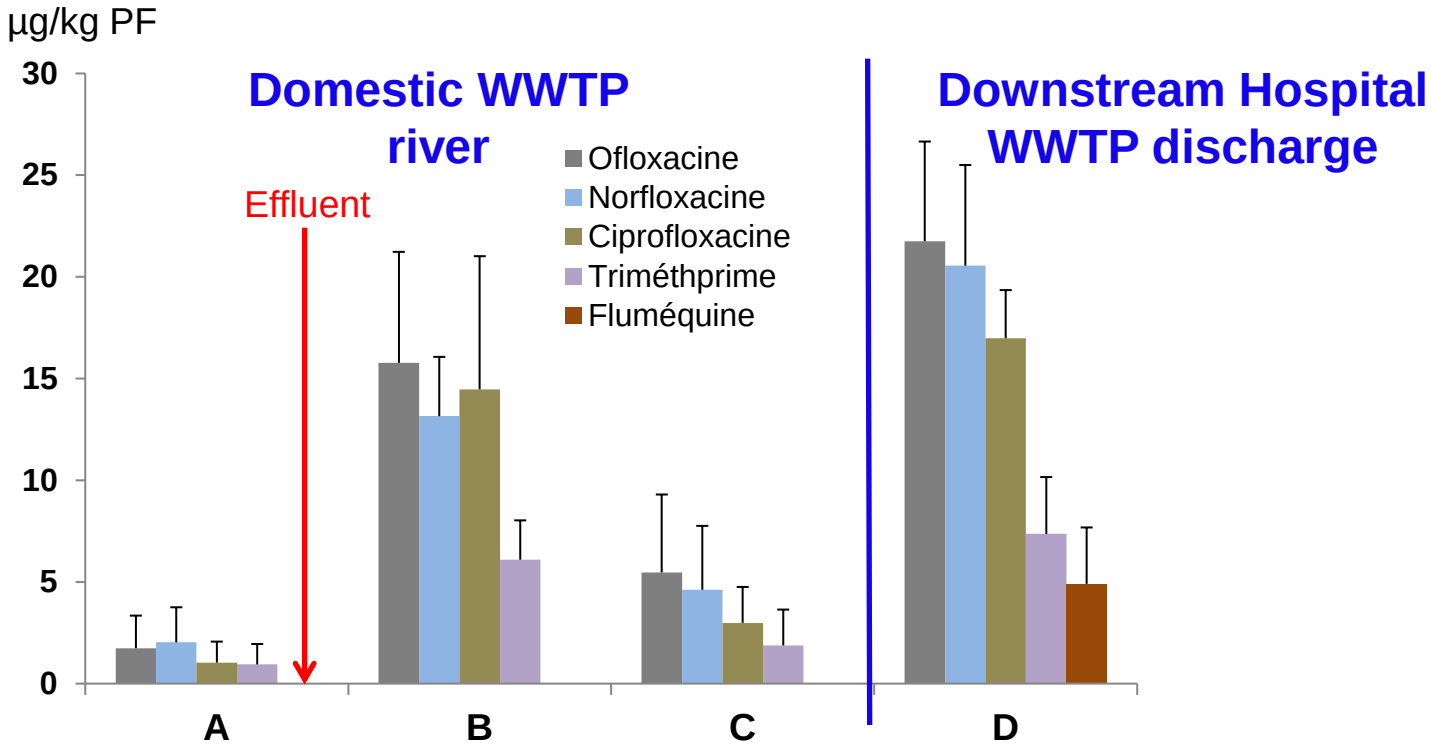




- 6 molecules detected including 5 fluoroquinolones
- Contents: Juveniles > Adults (more efficient elimination in adults?)
- Sulfamides, glycopeptides, macrolides : detected in water, not in Gammarus

Dinh et al, 2017

- Bioaccumulation in wild fish (stone loach)



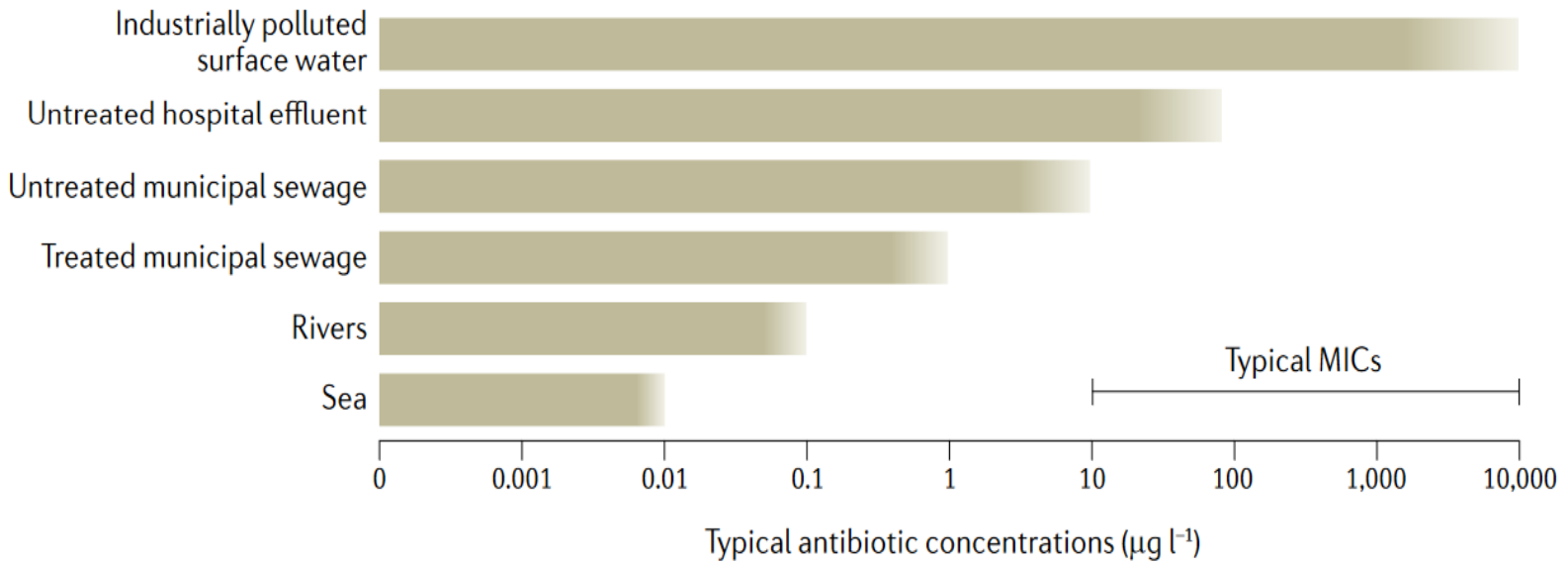
❖ 5 antibiotiques detected including 4 fluoroquinolones

❖ concentration in the same order than water contamination

Summary : ATB concentrations and AMR

- Concentrations vs minimal inhibitory concentrations MICs

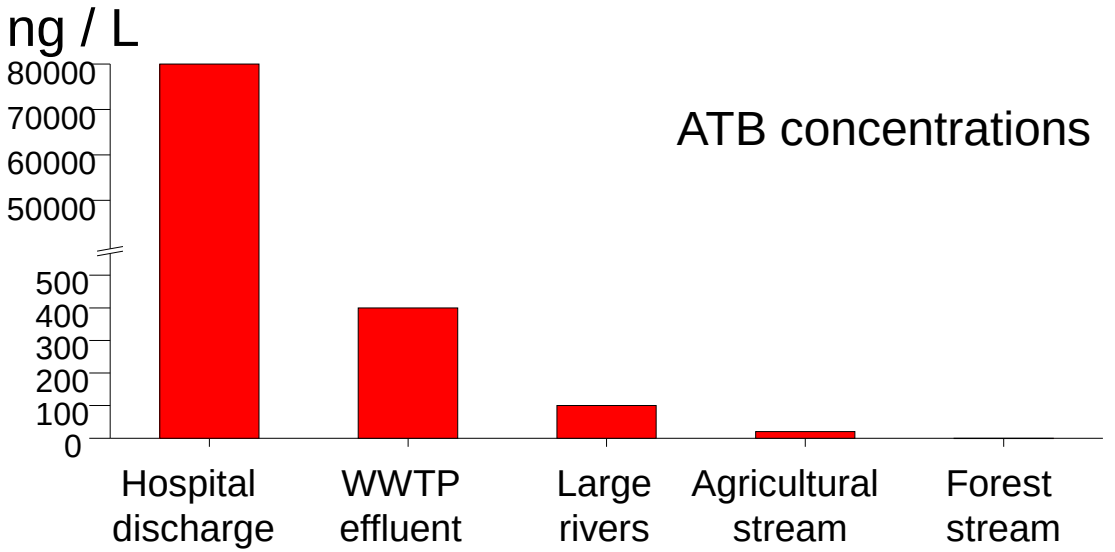
  => promote AMR

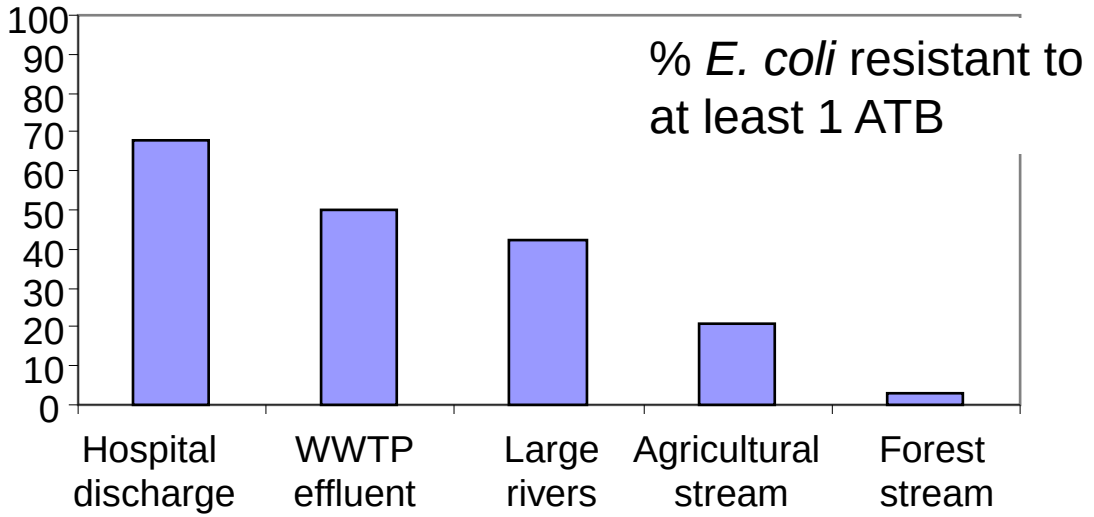


Larsson and Flach, 2022

Summary : ATB concentrations and AMR

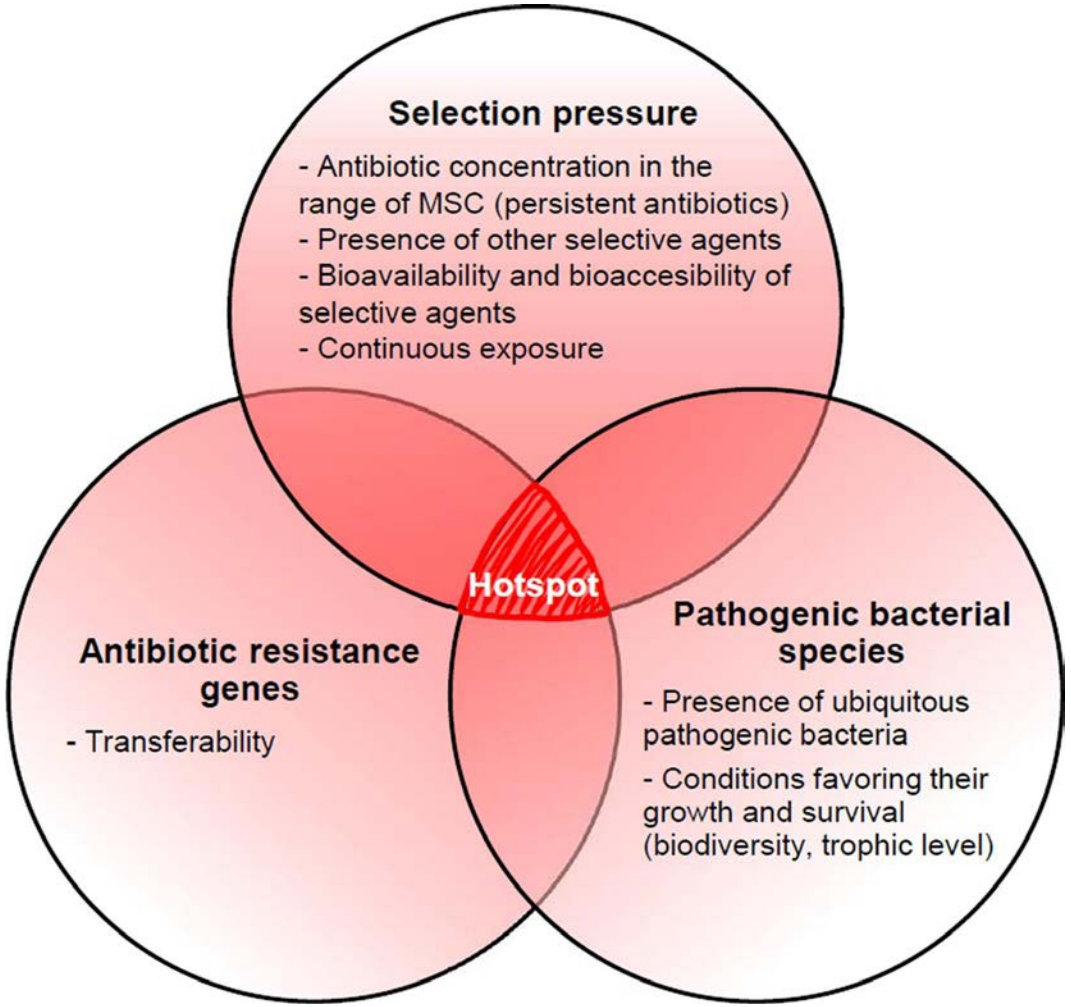
In the Seine river





Passerat et al., 2010

Summary : Hotspot



Haenni et al. *Environ Int 2022*

- Hotspot : 3 conditions
- Importance of studies about environmental fate of molecules
- Hotspot :
  - In regions with open defecation
  - In regions with pharma manufacturings sites (Asia)

Others pharmaceuticals and their impacts

Environmental consequences of the use of an anti-inflammatory drug on livestock

Diclofenac: anti-inflammatory drug

livestock: treatment of any inflammation, fever and/or pain associated with disease or injury.

In India, Vultures feed on the carcasses of these livestock

→ mortality: kidney disease.

The toxicity of this anti-inflammatory drug has been found in other raptors, in storks, cranes and owls

Oaks et al. (2004)

Impacts of veterinary products on coprophagous insects: consequences on the degradation of excreta in pastures

Livestock → excrement (dung))

↓ recycling organisms (Beetles,...)

Organic matter

Impacts of veterinary products on coprophagous insects: consequences on the degradation of excreta in pastures

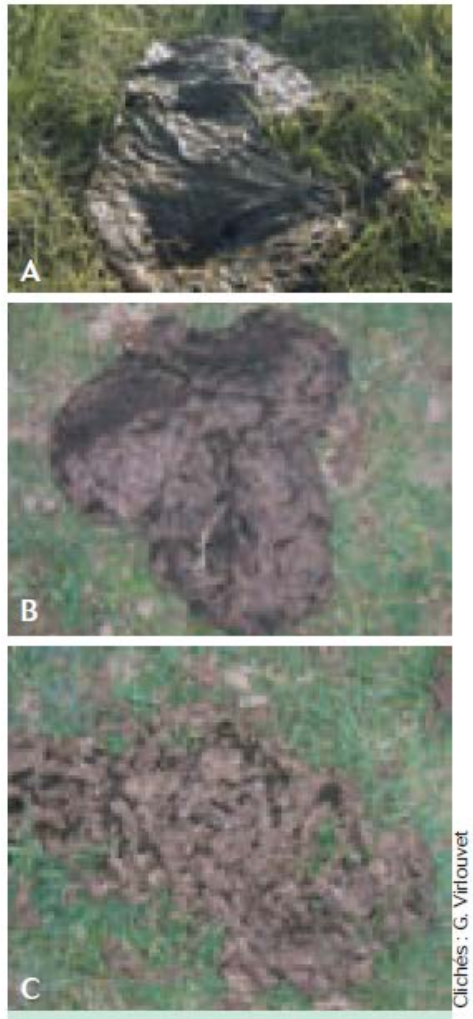
Livestock → excrement (dung))

↓ X recycling organisms

(Beetles,...)

Organic matter

Antiparasitic treatments: Ivermectin



Impact :

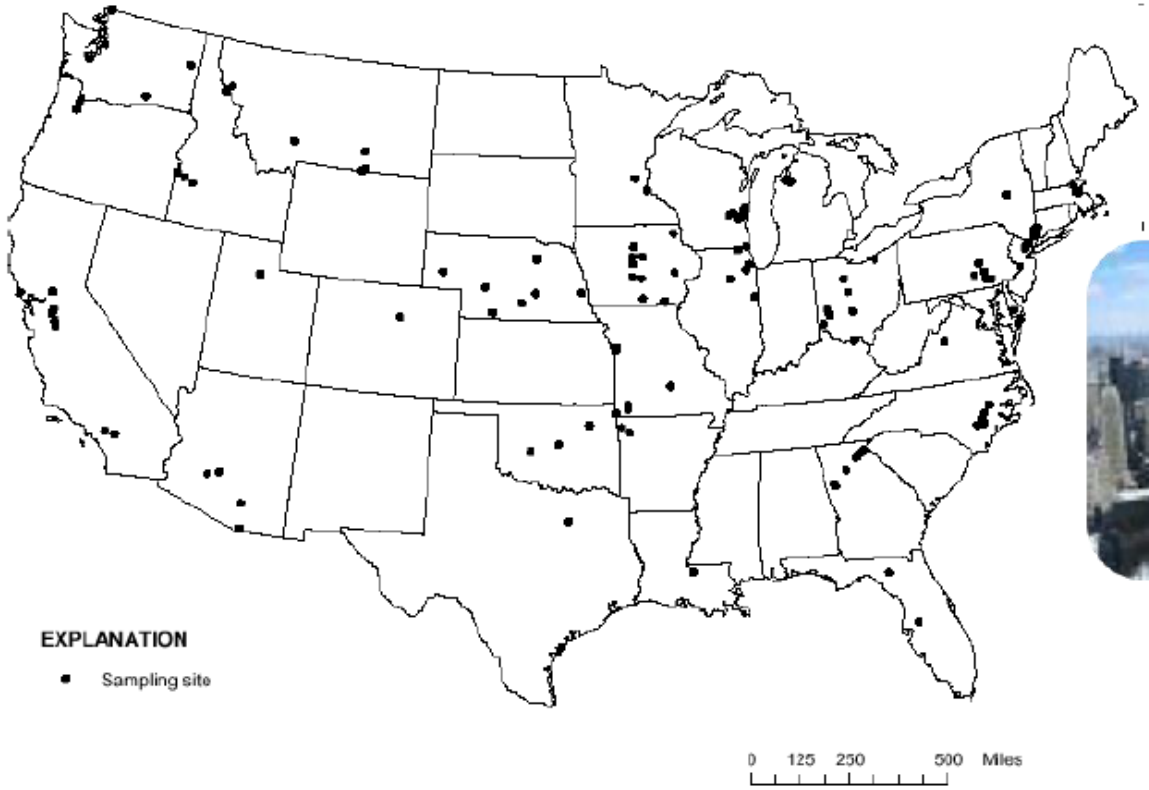
disappearance of recycling organisms

Accumulation of dung : fly outbreak and loss of grazing area

Others pharmaceuticals in aquatic environment

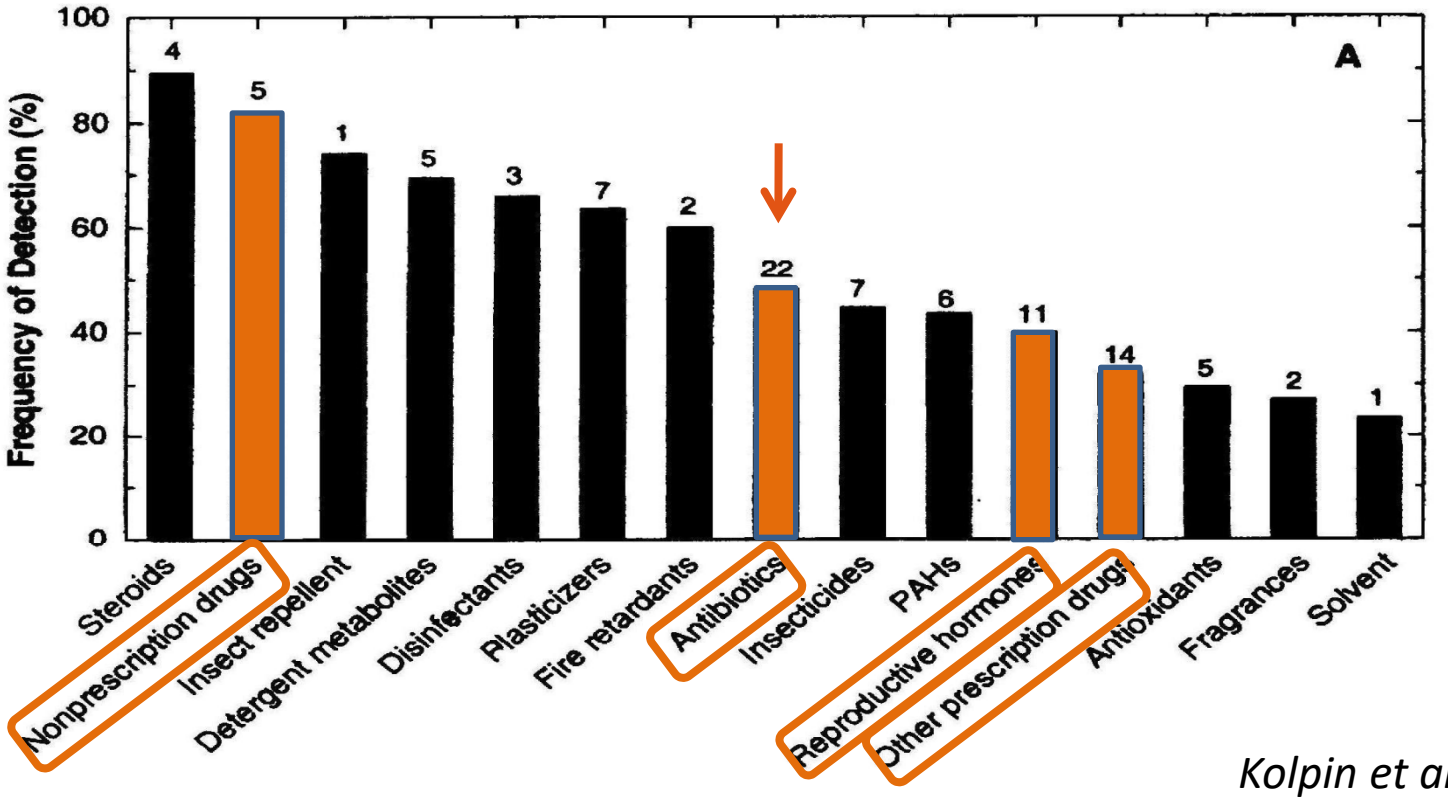
A 1st Study

Kolpin et al, 2002



139 streams
95 organic pollutants including 22 drugs + 31 human and / or veterinary antibiotics + 18 hormones and steroids

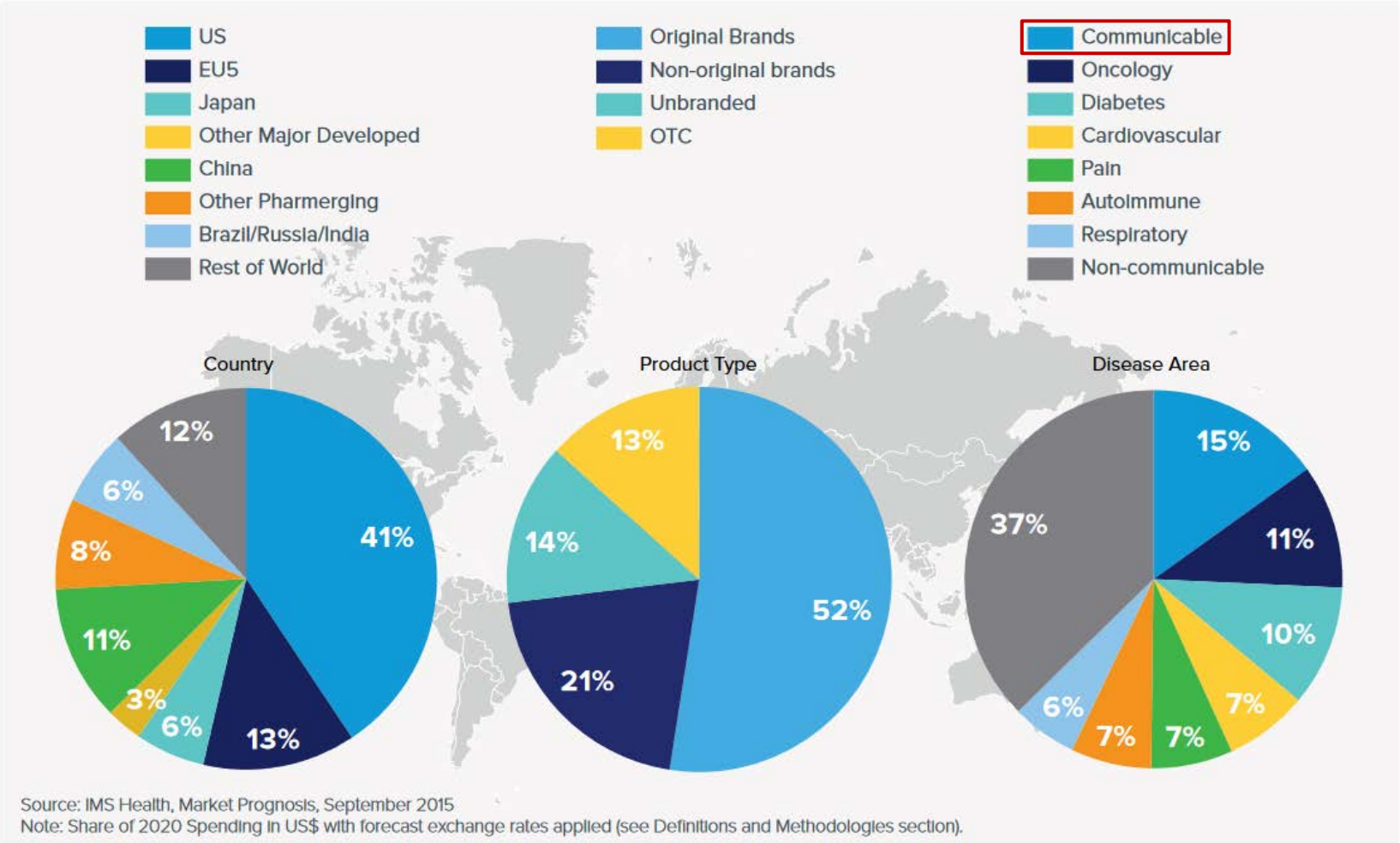
Place of drug among pollutants



Kolpin et al. 2002

Drug : wordwide consumption

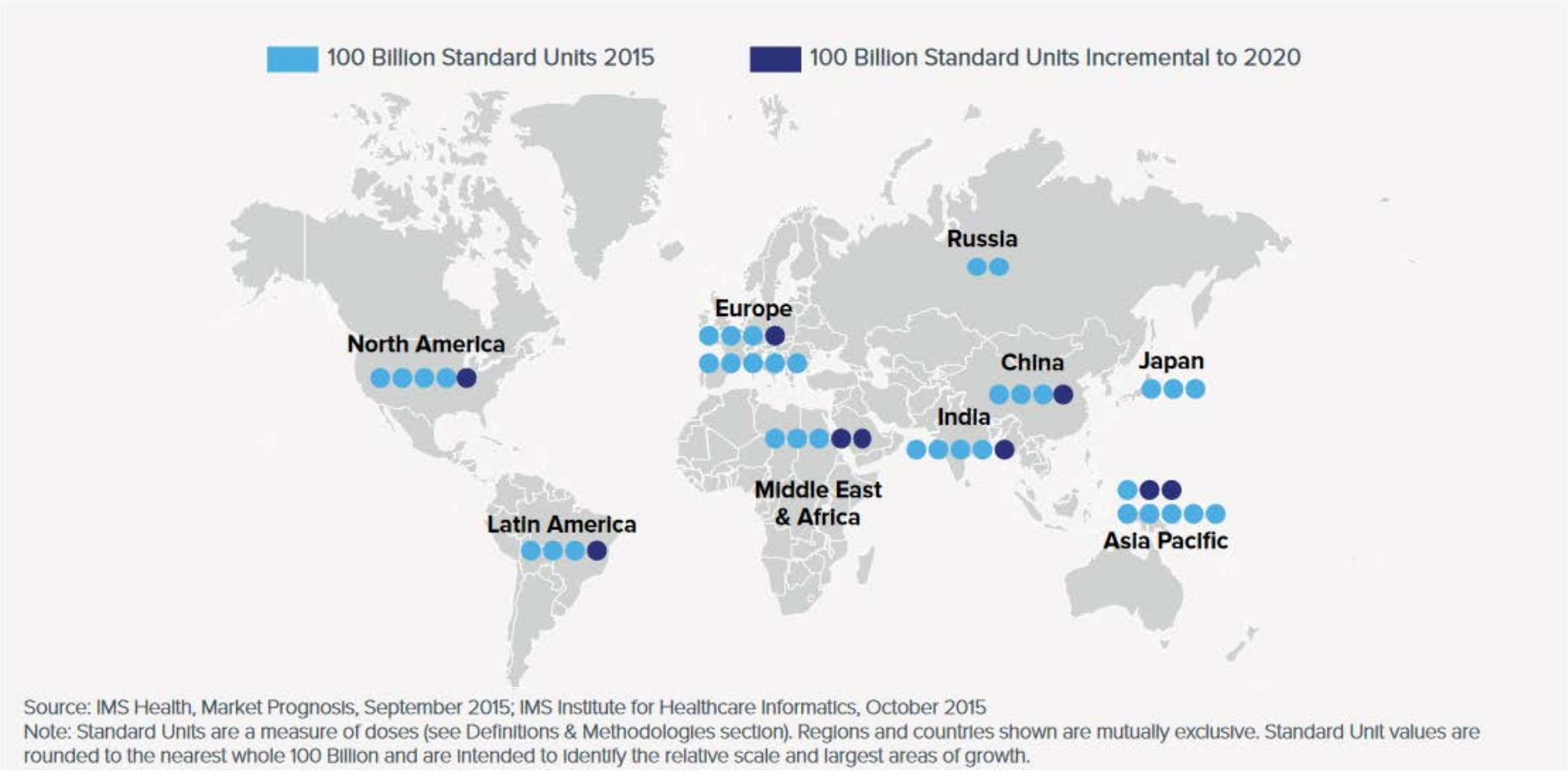
Exhibit 6: Medicine Spending in 2020 US$ by Geography, Product Type and Disease Area



Source: IMS Health, Market Prognosis, September 2015
Note: Share of 2020 Spending in US$ with forecast exchange rates applied (see Definitions and Methodologies section).

Global Use of Medicines in 2020. Report by the IMS Institute for Healthcare Informatics

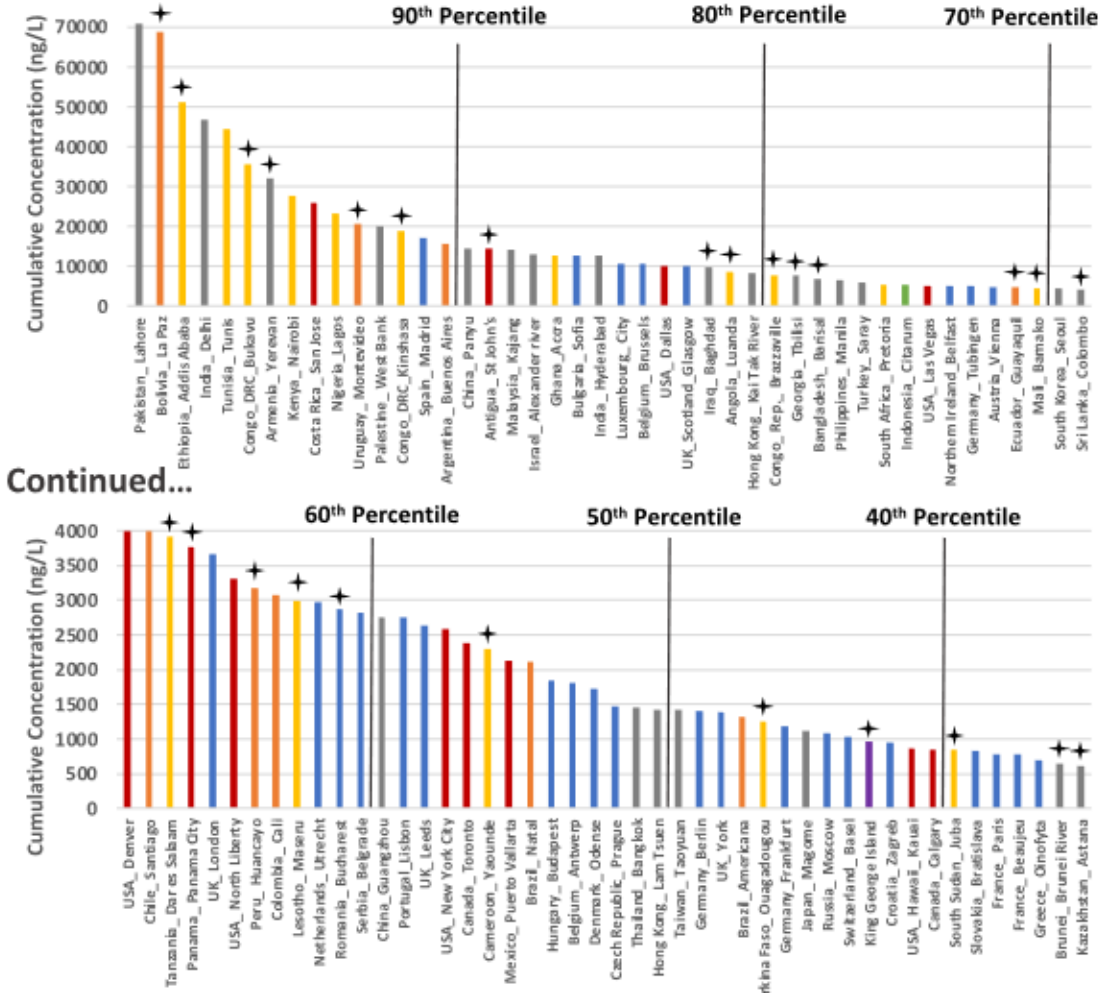
Drug : wordwide consumption



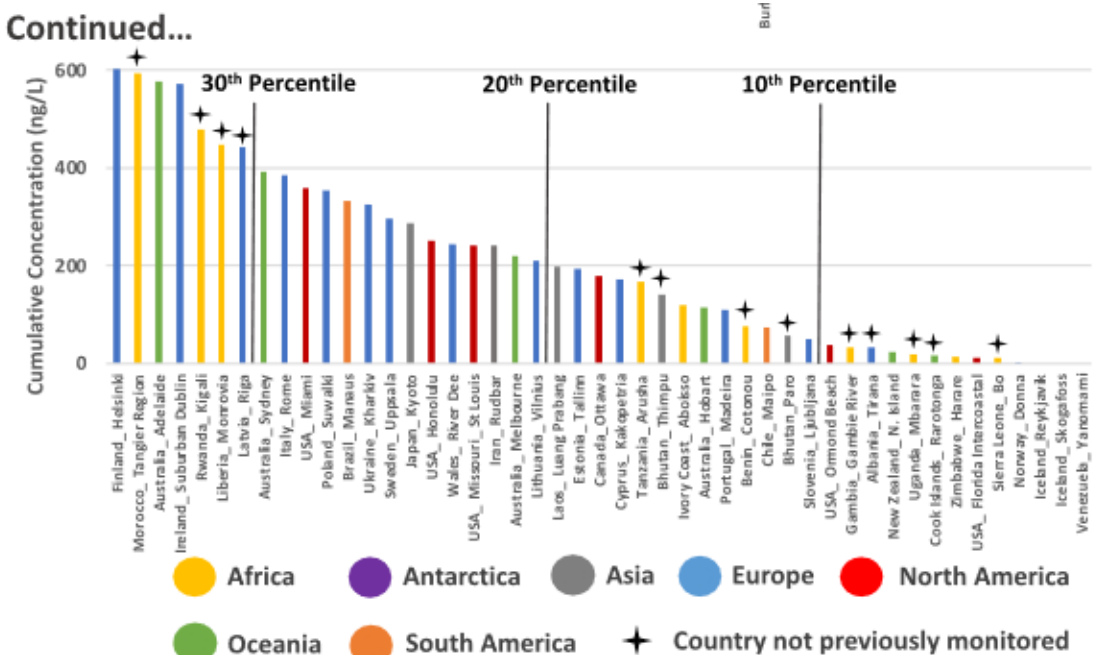
Global Use of Medicines in 2020. Report by the IMS Institute for Healthcare Informatics

Pharmaceutical pollution in the world's rivers

Wilkinson et al. 2022

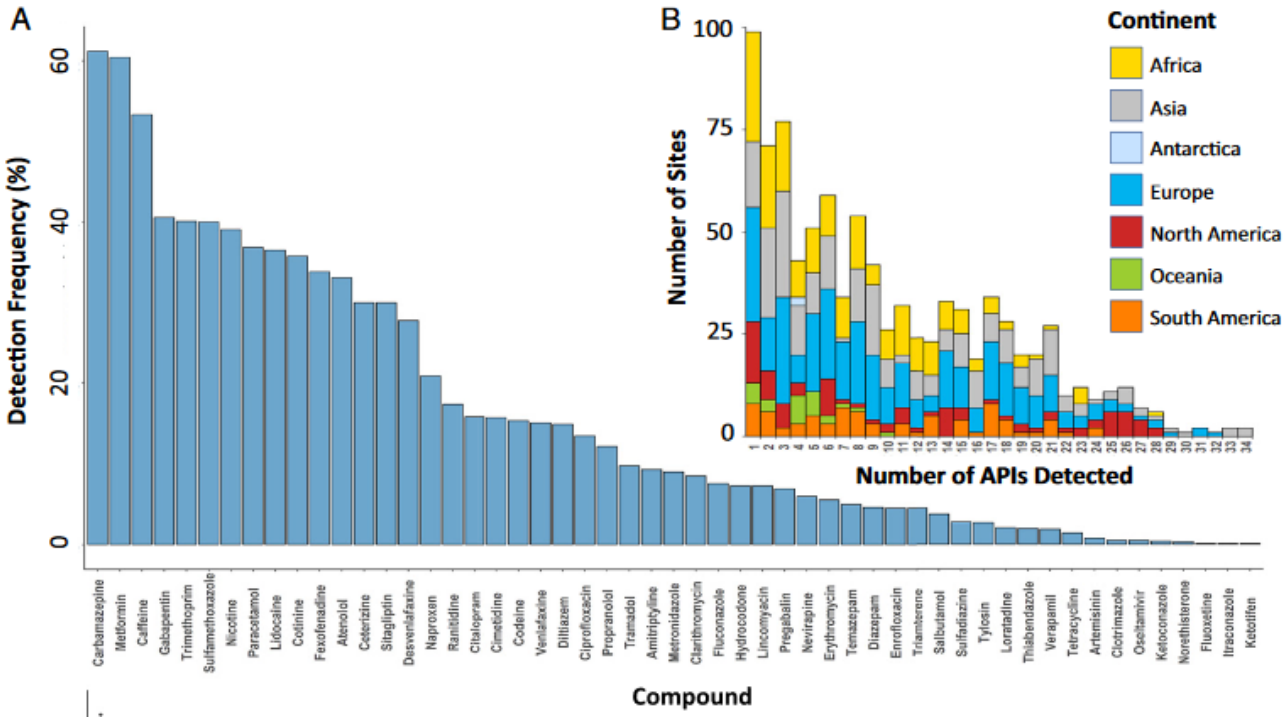


- Sum of Active Pharmaceutical ingredients
- 1052 sampling sites, 104 countries, 137 rivers, 61 APIs
- Pakistan >>> Venezuela -Yanomami (no use)
- African – Asia : most contaminated



Pharmaceutical pollution in the world's rivers

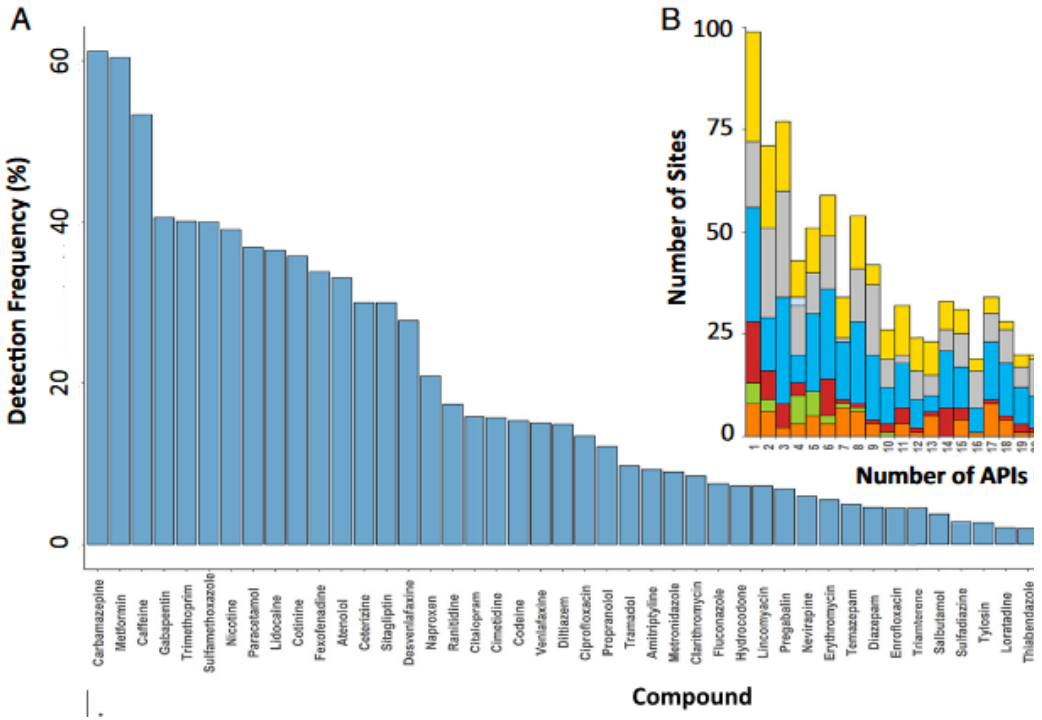
Wilkinson et al. 2022

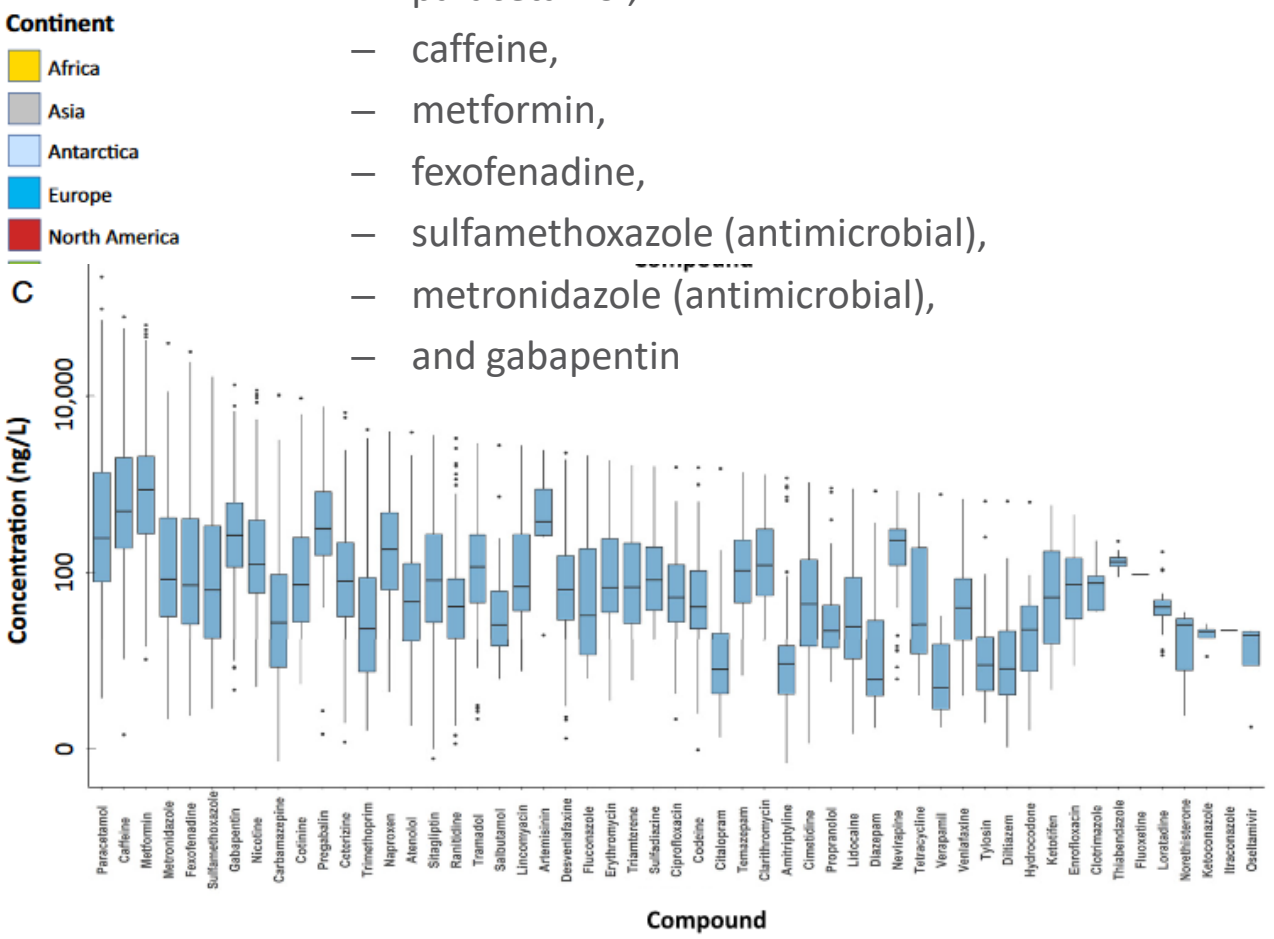


- 53/61 APIs detected once
- 15 compounds > 20% detection
- > 40% Carbamazepine, Metformine, Caffeine
- Trimethoprime and sulfamethoxazole 1st ATB (40%)

Pharmaceutical pollution in the world's rivers

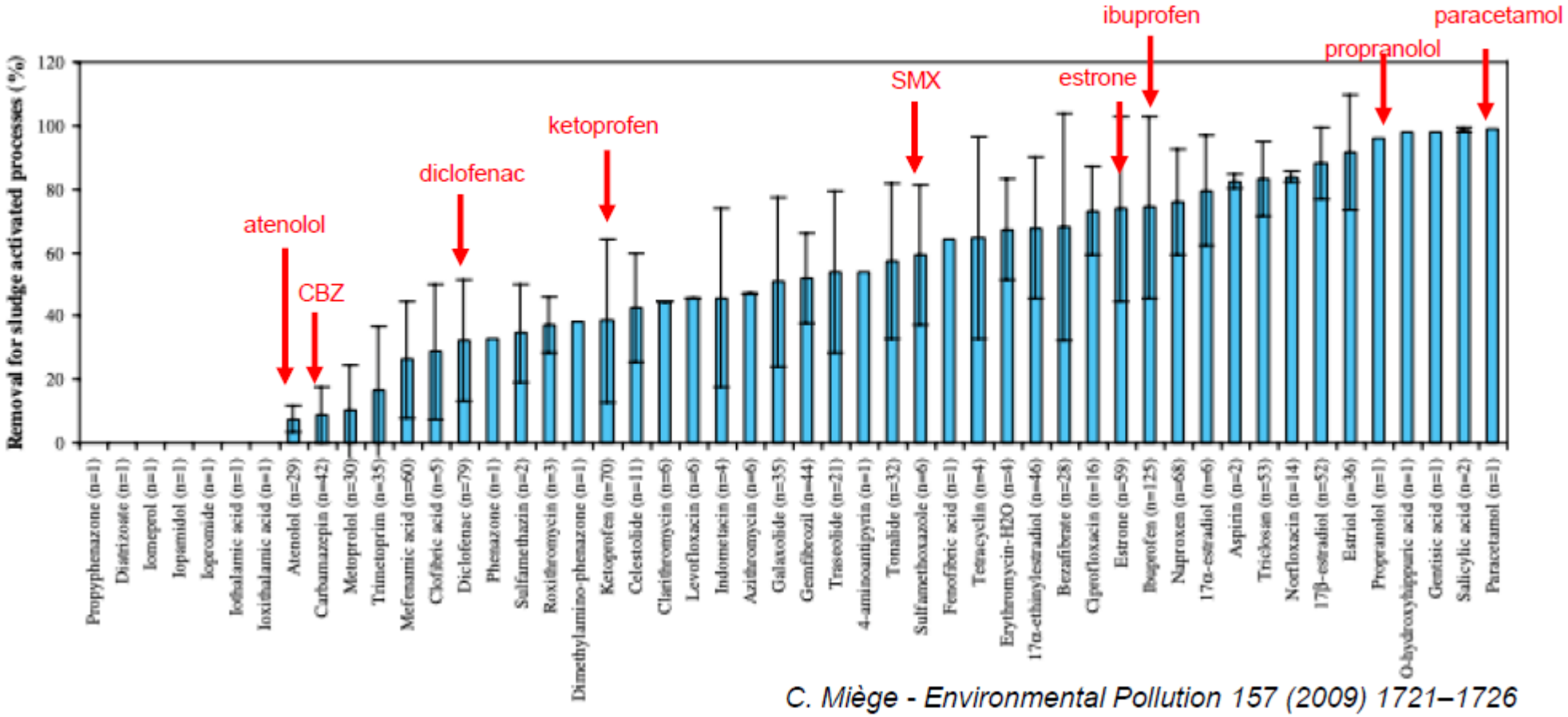
Wilkinson et al. 2022





- highest concentrations :
  - paracetamol,
  - caffeine,
  - metformin,
  - fexofenadine,
  - sulfamethoxazole (antimicrobial),
  - metronidazole (antimicrobial),
  - and gabapentin

Example of removal in WWTP



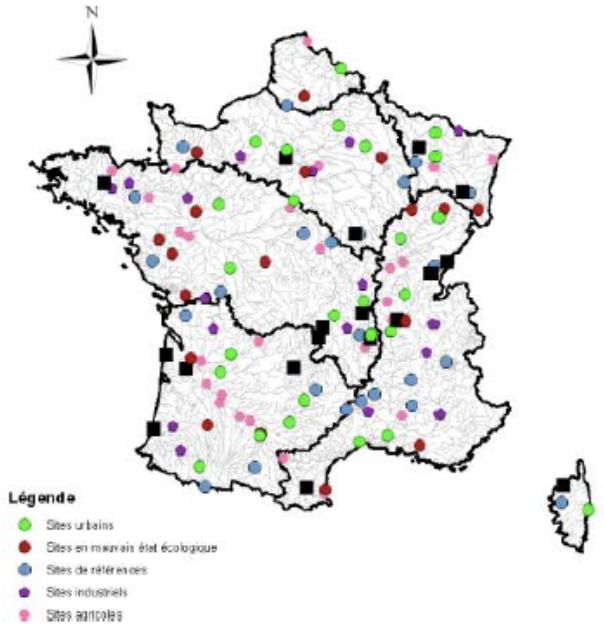
C. Miège - Environmental Pollution 157 (2009) 1721–1726

As ATB, removals in WWTP of pharmaceutical are not 100%

Study on emerging contaminants in French waters

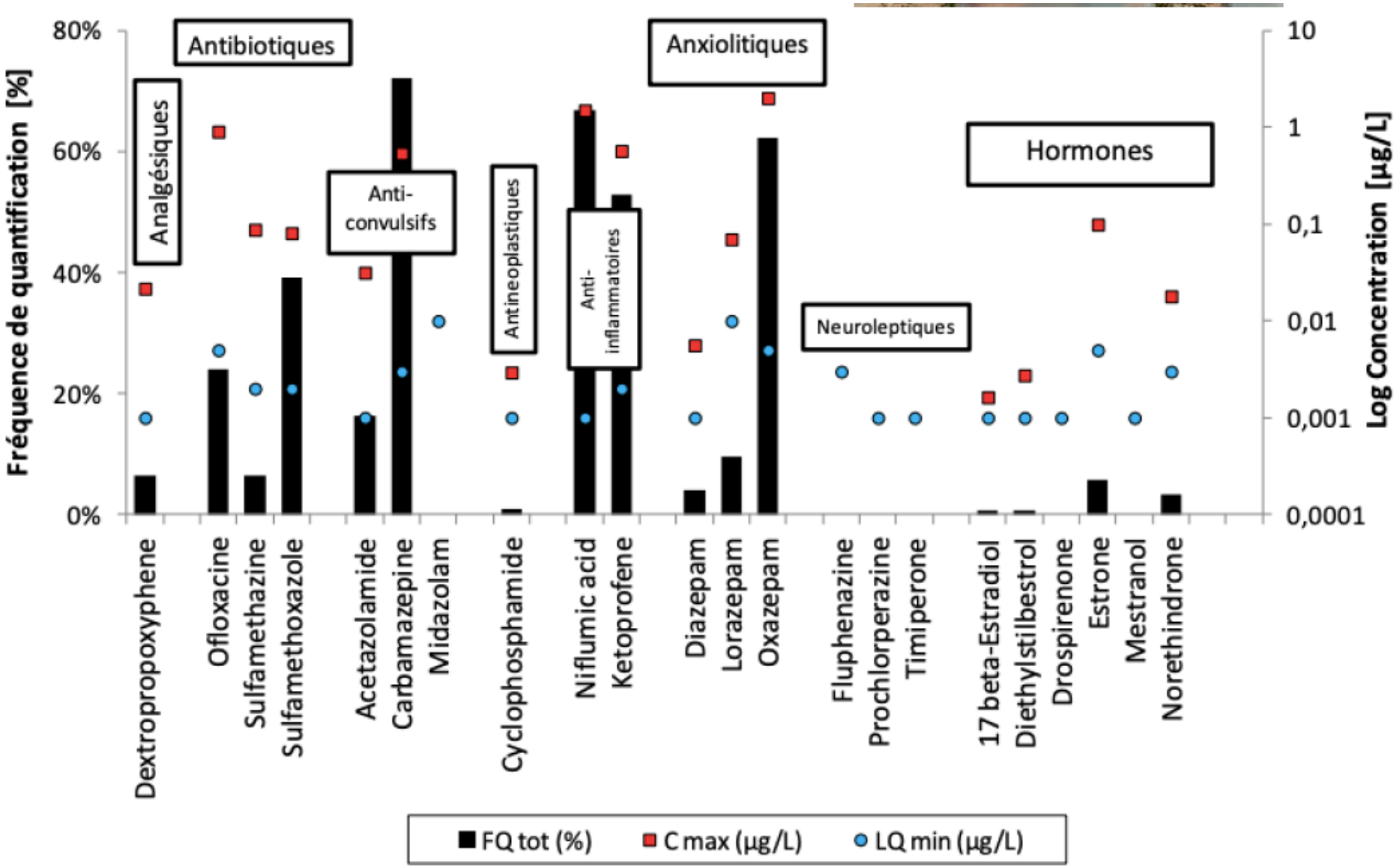
- ONEMA & INERIS, 2014
- Three campaigns: spring, summer, fall
- 158 locations in France
- 22 pharmaceuticals from 10 therapeutic families
- 75% pharmaceuticals quantified at least once in water and 52% in sediments

Catégorie	Paramètre	FQ C1	FQ C2	FQ C3
Antibiotique	Sulfamethoxazole	30,1%	50,9%	36,3%
	Ofloxacine	27,4%	25,5%	19,5%
	Sulfamethazine	16,8%	1,8%	0,9%
Anticonvulsant	Carbamazepine	85,0%	66,4%	64,6%
	Acetazolamide	17,4%	20,4%	11,4%
Anti-inflammatoire	Ketoprofene	54,9%	50,0%	54,0%
	Acide niflumique	55,1%	73,2%	71,1%
Anxiolytique	Diazepam	2,8%	5,6%	3,5%
	Oxazepam	69,0%	75,5%	42,5%
	Lorazepam	10,8%	9,1%	8,9%
Hormone et steroide	17 beta-Estradiol	0%	0,9%	0,9%
	Estrone	16,8%	0%	0%
	Diethylstilbestrol	0%	1,9%	0%
	Norethindrone	8,0%	1,8%	0%

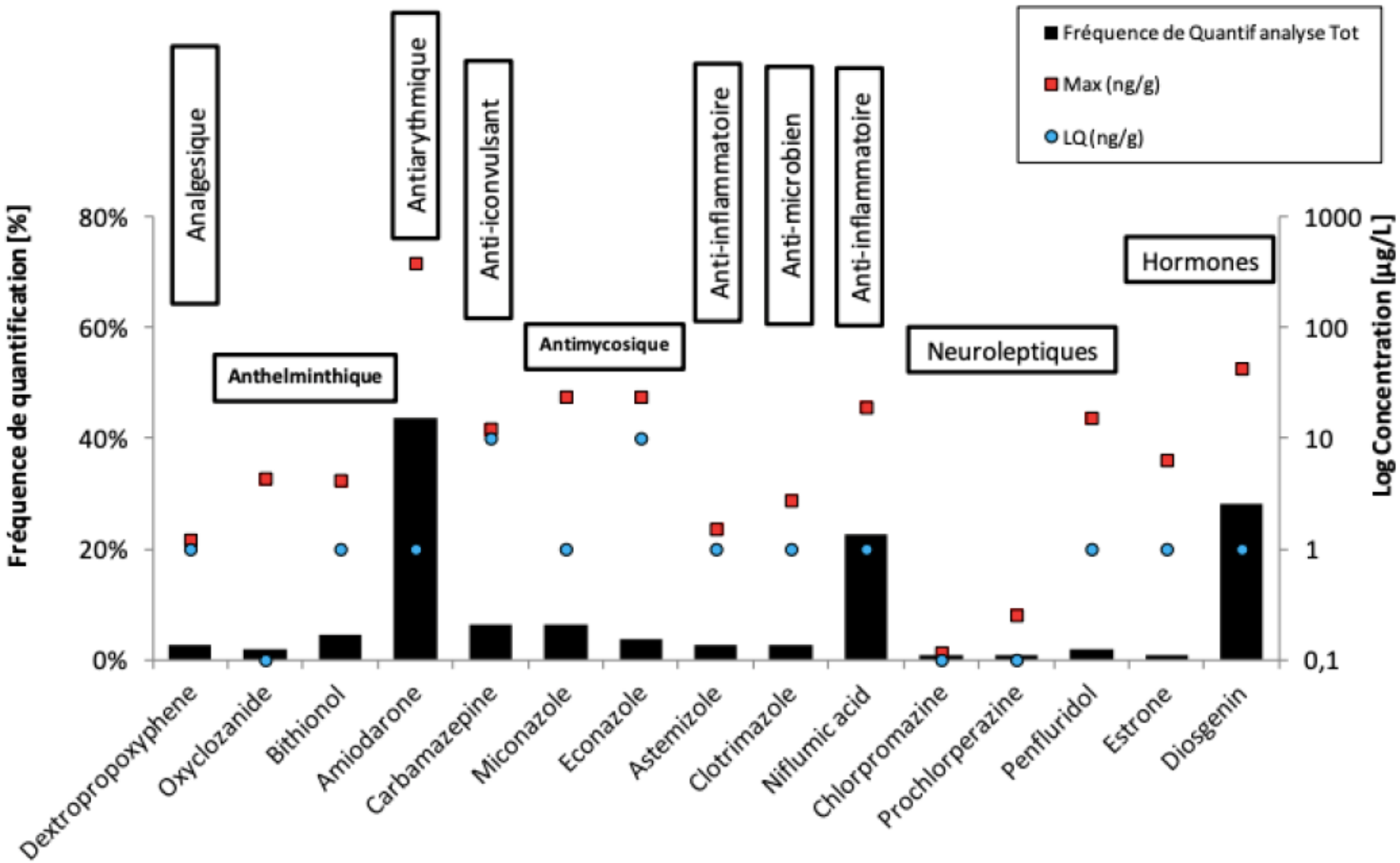


No difference between sites except for pesticides, less present in industrial sites

Onema Ineris study – surface waters

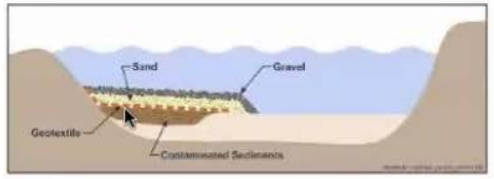


Onema Ineris study – sediments



Persistence in sediments

Antibiotics accumulate in the sediments from their market authorization date



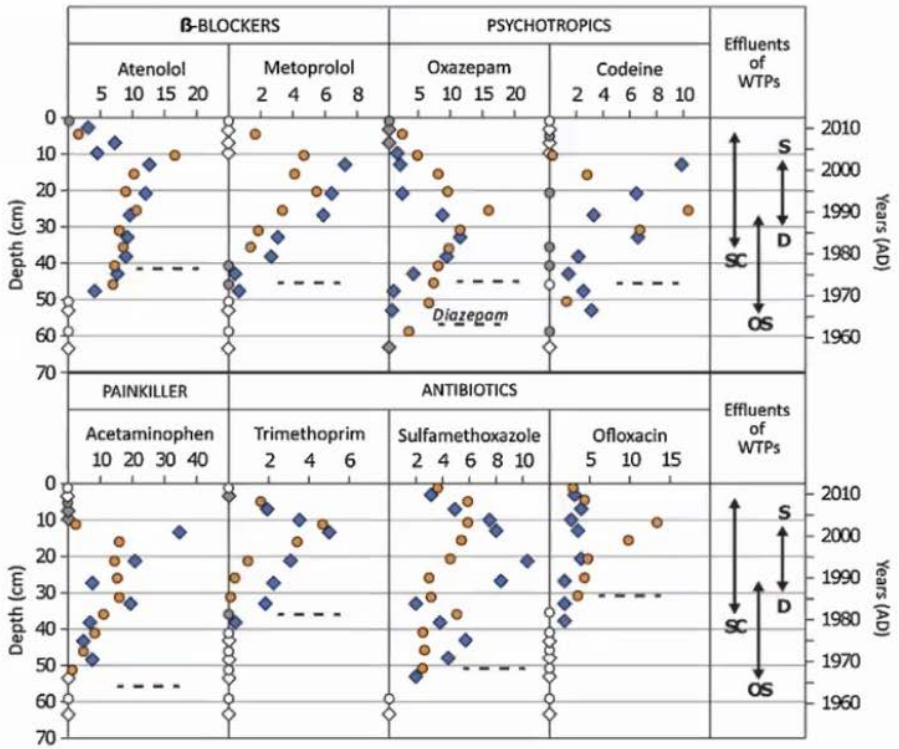


Fig. 4. Distribution of PPs along core LOI13-2 (orange circles) and core LOI13-6 (blue diamonds) according to depth and years (age-depth model performed on core LOI13-2). Concentrations are expressed in ng g^{-1} of dried sediment. Grey markers indicate concentrations below LOQ whereas empty markers indicate concentrations below LOD. The dashed line refers to the market authorization date (found on the ANSM website, www.ansm.fr) for each compound unless otherwise specified. The evolution of WTP effluents feeding the Dhuy River is indicated on the right-hand side of the figure (Fig. 1 and Table 1).

Thiebault et al. 2017

French National campaign for the analysis of drug residues in water 2009-2010

- 45 compounds (with QLs of 1 to 50 ng/L)
- 285 raw water samples and 285 treated water samples:

- Majority of molecules not quantifiable,
- No excessive concentrations

- Raw water :
  - 2/3 groundwater, 1/3 surface water
  - 35% surface water and 70% groundwater <LQ
  - 30 molecules detected 1 time, of which 14 <LQ
  - Max Conc: 400 ng/L (1% samples)
  - Majority Cumulative Conc < 25 ng/L
  - Oxazepam, paracetamol, carbamazepine

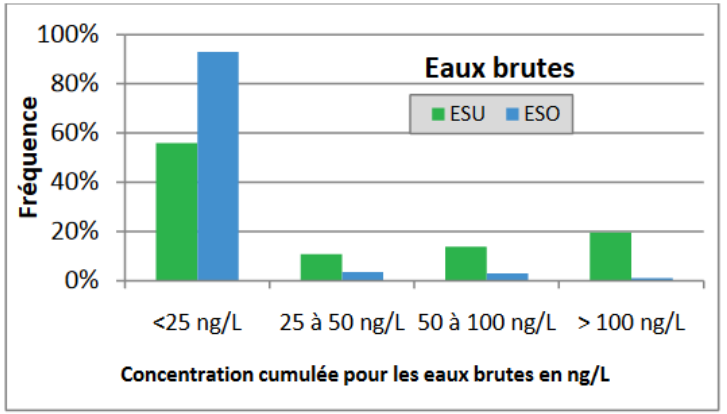


Figure 9 : Niveaux de concentrations cumulées de résidus de médicaments dans les eaux brutes

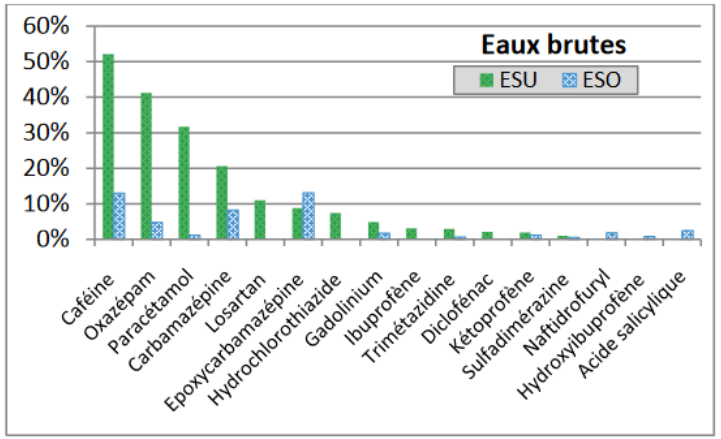


Figure 2 : Occurrence des différentes molécules quantifiées dans les ressources en fonction de l'origine de l'eau

French National campaign for the analysis of drug residues in water 2009-2010

- Treated water :
  - 26 molecules not detected
  - 19 quantified 1 time, 5 < LQ
  - 25% with 1 to 4 molecules quantified
  - Most frequently detected molecules: epoxycarbamazepine, carbamazepine, oxazepam and hydroxyibuprofen
  - > 90%, cumulative conc < 25 ng/L
  - < 5% cumulative conc < 100 ng/L; max: 131 ng/L (1 sample)

- [conc] raw water > treated water: treatment efficiency

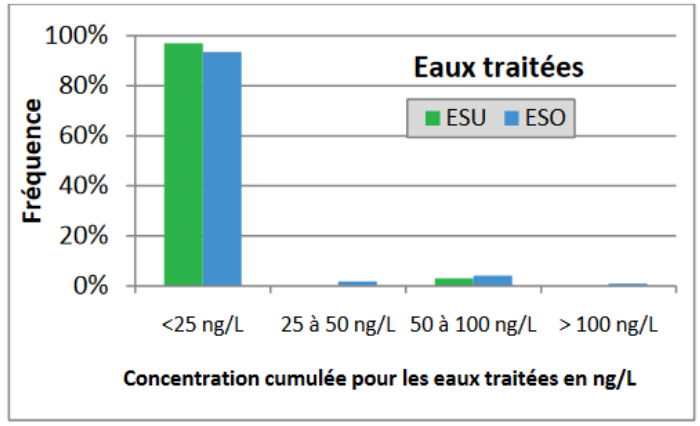


Figure 10 : Niveaux de concentrations cumulées de résidus de médicaments dans les eaux traitées

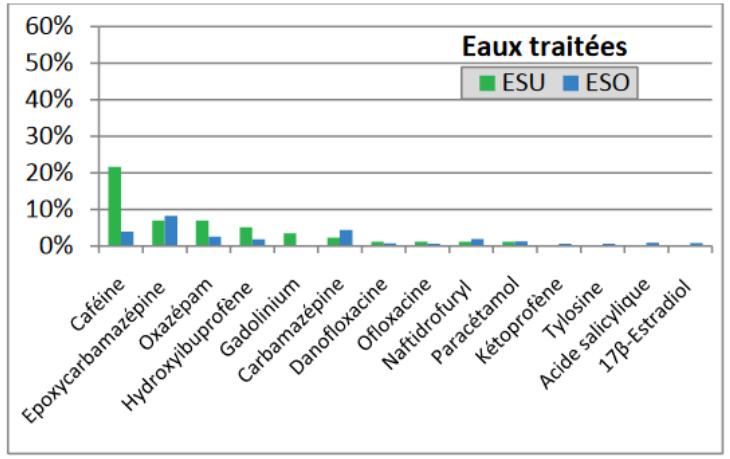


Figure 3 : Occurrence des différentes molécules quantifiées dans les EDCH en fonction de l'origine de l'eau