From obscurity to “superbug”: the rise of *Clostridium difficile*

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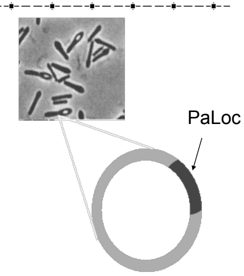
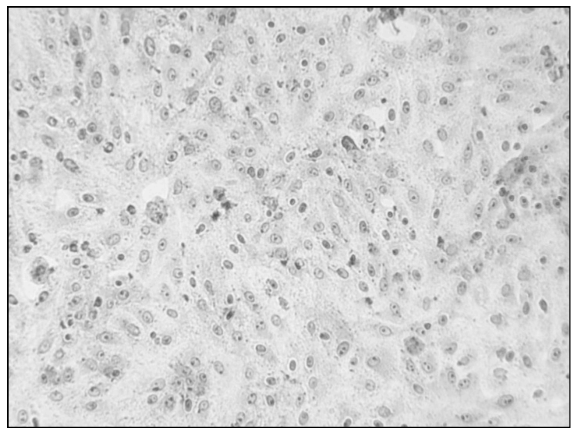
Background

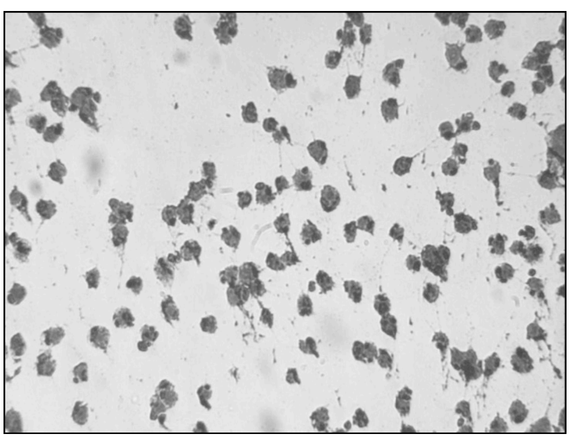
- *Clostridium difficile* – an anaerobic Gram +ve bacillus
- *C. difficile* disease gained prominence because of renewed interest in anaerobic bacteria in the 1960s and 70s
- specific anti-anaerobe drugs had been developed, e.g. clindamycin
- clindamycin-associated diarrhoea became a real problem in some hospitals in the USA
- outbreaks of pseudomembranous colitis
- cause elucidated in 1978

C. difficile-associated disease

- *C.difficile* the most common cause of diarrhoea in hospital patients
- Produces at least 2 major toxins:
  - toxin A (an enterotoxin)
  - toxin B (a cytotoxin)
- 3rd “binary” toxin

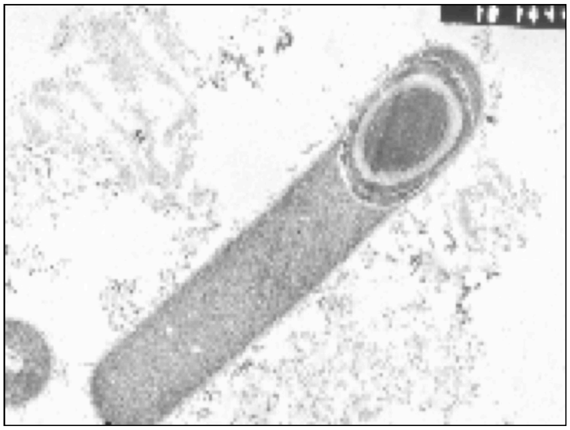






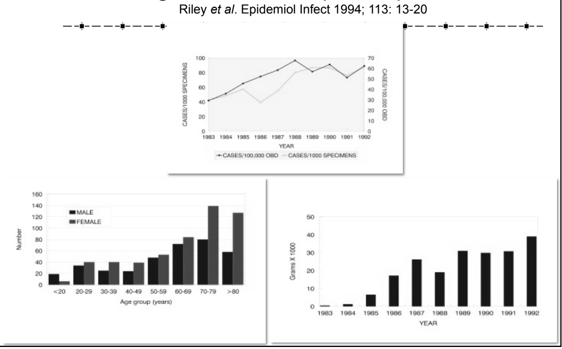
What are the major risk factors for *C.difficile* ?

- Exposure to the organism
- Exposure to antibiotics - particularly clindamycin and extended spectrum cephalosporins (until now)
- Old age



CDAD at SCGH 1983-92, rates, by age and gender, and cephalosporin use

Riley *et al.* Epidemiol Infect 1994; 113: 13-20



Canadian outbreak in Quebec (CHUS)

(Pepin *et al.* CMAJ 2004;171:466-72)

- In late 2002, increase in fulminant *C.difficile* colitis → emergency colectomy.
- Retrospective review of all cases 1991-2003
- Rates ↑ from 35.6/100,000 pop. in 1991 to 156.3 in 2003.
- In ≥65 years age group ↑ from 102 in 1991 to 866.5 in 2003.
- Complications ↑ from 7.1% in 1991 to 18.2% in 2003 ($p<0.001$)
- Death within 30 days of diagnosis ↑ from 4.7% (8/169) in 1991 to 13.8% (59/390) in 2003 ($p<0.001$)

Pepin *et al.* CMAJ 2004;171:466-72

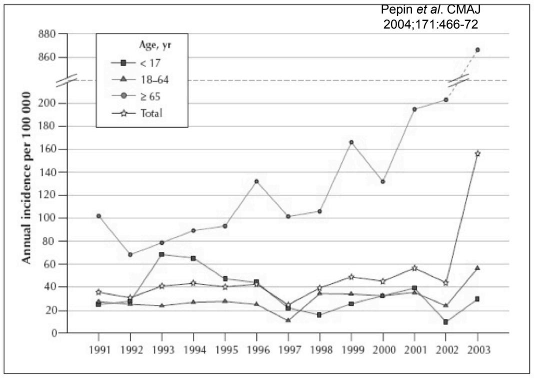


Fig. 1: Annual incidence (per 100 000 population) of *Clostridium difficile*-associated diarrhea (CDAD) in Sherbrooke, Que., 1991–2003.

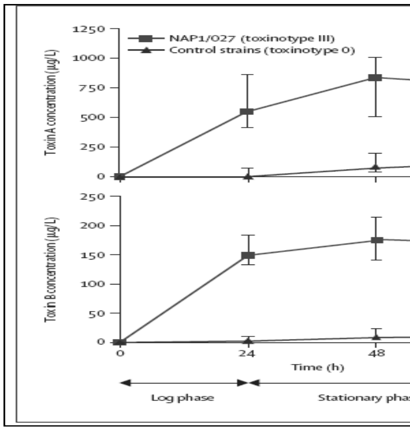
C.difficile Quebec 2000-2003

Year	Cases	Deaths	%
2000	3262	398	12
2001	3675	562	15
2002	4097	661	16
2003	7004	1270	18

From Obscurity to Superbug: The Rise of Clostridium difficile

Dr. Thomas Riley, University of Western Australia

A Webber Training Teleclass



Warny *et al.* Lancet 2005;366:1079

Resistance of current BI/NAP1 isolates to clindamycin and FQs compared with current non-BI/NAP1 isolates and historic BI/NAP1 isolates

No. (%) Intermediate or Resistant to:	Current BI/NAP1 Isolates n=24 (%)	Current non-BI/NAP1 Isolates n=24 (%)	*P-Value for BI/NAP1 vs. Non-BI/NAP1 Isolates*	Historic BI/NAP1 Isolates n=14 (%)	P-*Value for Current vs. Historic BI/NAP1 Isolates*
Clindamycin	19 (79)	19 (79)	1.0	10 (71)	0.7
Levofloxacin	24 (100)	23 (96)	1.0	14 (100)	1.0
Gatifloxacin	24 (100)	10 (42)	<0.001	0	<0.001
Moxifloxacin	24 (100)	10 (42)	<0.001	0	<0.001

McDonald *et al. N Engl J Med.* 2005; 353: 2433-2441

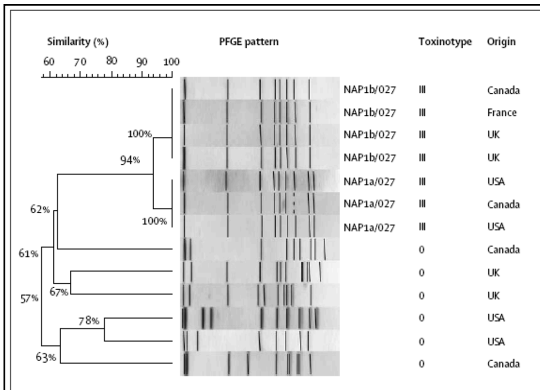
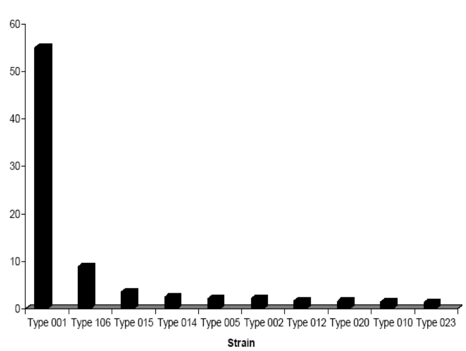


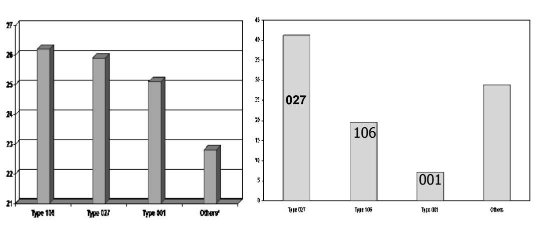
Figure 1: PFGE analysis of *C difficile* study isolates from various geographical locations

Warny *et al.* Lancet 2005;366:1079

Figure 5: PCR types of *C. difficile* in hospital patients in England and Wales: referrals to ARL 1995- 2003



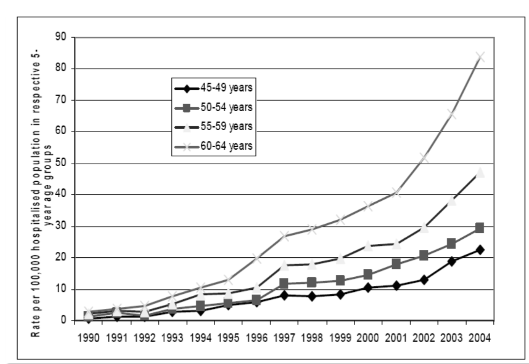
England distribution of PCR ribotypes 2005/6 to 2007/8 as percentages



2005-6 (n=881)

2007-8* (n=677)

* Brazier et al. *Eurosurveillance* Vol.13;4. October 2008



Clostridium difficile **rates from laboratory reports in people 45-64 years of age in England (excludes North West, South East and London regions) (Health Protection Agency, 2006).**

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INTERNATIONAL EXPRESS Tuesday May 6, 2008 15

Superbug kills one person an hour

SUPERBUG clostridium difficile now claims a life every hour, according to shocking new evidence.

The C. diff bacteria now kills four times as many people as MRSA and is showing signs of resistance to antibiotics.

Startling statistics also show that in Britain it is responsible for the deaths of 10 times more hospital patients aged over 65 than in any other country.

The research also casts doubt on government figures that show C.diff infections in England fell eight per cent in the last three months of 2007. Research suggests the supposed fall is due to a change in the way cases are counted, and that true figures show a substantial increase in infection of between 16 and 35 per cent during that period.

Professor Richard James, who is in charge of Nottingham University's centre for investigating hospital infections, said: "The figures for C. diff show more than 50 per cent of hospital trusts have a rate of infection that's more than 10 times that of any other country.

"If you look at the over-65s, which is the group where there are more deaths, then we have more cases there and therefore more deaths in that age group, than any country in the world by a factor of 10."

His remarks are based on a draft report on infection for the Department of Health, and while the figures are based on English acute NHS trusts, he says they are relevant to the whole of the UK. A Freedom of Information survey of every acute trust and health board in Britain carried out by TV's Panorama, got replies from 170 trusts – 83 per cent of the total.

In 95 of the trusts that replied, the bed occupancy rates were more than 85 per cent. Prof James said: "Experts would say if you go above 85 per cent bed occupancy that is not conducive to good control of infection measures."

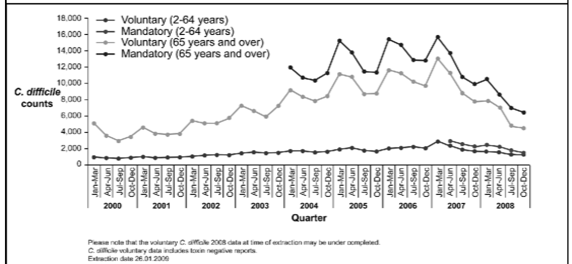
The survey also reveals that 94 per cent of hospitals now put alcohol hand gels outside their infection wards, and while this destroys MRSA, it does not combat C. diff, though soap and water does offer protection.

Scientists are also worried that the superbug is becoming resistant to Metronidazole, one of the only two antibiotics that can destroy it. Although the Department of Health has put £270million into a new initiative within hospitals and £57million towards deep cleaning, experts say more money is needed for research.

One of the worst outbreaks was at the Maidstone and Tunbridge Wells Trust in Kent, where around 90 patients died in 2005-6.

Health minister Ann Keen said: "One case of avoidable infection is one too many and I challenge the NHS to make full use of their resources to eradicate avoidable infections."

Quarterly counts of *C. difficile:* comparison of mandatory and voluntary quarterly reporting



Courtesy Andrew Pearson

In Australia

- We have CDT+ strains
- We have hyper-toxin A & B producers
- No evidence of widespread quinolone resistance (~2%)
- Currently no widespread evidence of all 3 features in one strain – no PCR ribotype 027
- First case reported last year (Riley *et al*. Med J Aust 2009) – current outbreak in Melbourne
- Continued surveillance required – but how?

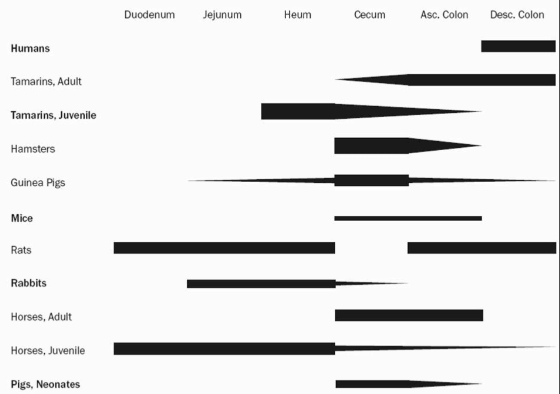
What is driving this apparent epidemic?

- Aging population
- New fluoroquinolone use
- Gastric acid suppressant use
- ?Animal reservoir, pigs/cattle for example

Is *C. difficile* infection part of a zoonoses?

Potential animal reservoirs of *C. difficile*

- Camels
- Seals
- Cattle
- Snakes
- Donkeys
- Deer
- Horses
- Hares
- Guinea pigs
- Native cats
- Kodiak bear
- Domestic cats
- Dogs
- Quokkas
- Numbats
- Antelopes
- Pigs
- Hamsters



Keel et al. 2006

C. difficile in companion animals

- 21 cats and 60 dogs at 2 veterinary clinics
- 32 (39.5%) yielded *C. difficile*
- cats (38% positive) vs dogs (40% positive)
- clinic 1, 61% positive vs 17.5%, clinic 2
- positive animals receiving antibiotics
- 62% of environmental sites positive

Riley *et al. Epidemiol Infect* 1991: 107: 659-665

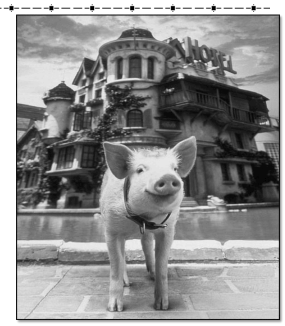
Relationship between human and animal strains of *C. difficile*

- Total of 116 isolates of *C. difficile*
- Pets (26) and humans (37)
- Veterinary clinics (33) and hospitals (20)
- REA and ribotyping
- Good correlation between vet clinics & pets
- Good correlation between humans & hospital
- No relationship between pets and humans

O'Neill *et al. Epidemiol Infect* 1993; 111: 257-264

C. difficile in pigs

- First described over 20 years ago in gnotobiotic pigs
- PMC then described in conventional pigs
- Early this century outbreaks of CDAD in 5d old piglets in USA - high mortality (16%)
- Since 2000, *C. difficile* the major & most common cause of enteritis in neonatal piglets in USA
- Most producers didn't realise they had a problem – now over 90% herds affected



C. difficile in pigs

- Apart from mortality, affected piglets are 10% under weight when ready for market leading to significant losses in income
- Piglet isolates mainly PCR ribotype 078 (~80%) – this is/ was an uncommon human ribotype
- PCR ribotype 078 increasing in USA & The Netherlands
- No data from other big pig growing countries such as Denmark and China
- Preliminary work in WA indicates some affected herds but mainly being ignored by the industry!

C. difficile in cattle

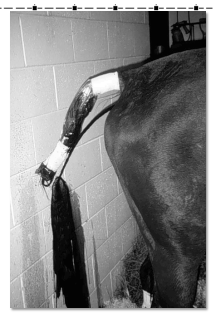
- Neonatal calf diarrhoea a common illness (10%) in calves
- Many cases idiopathic
- *C. difficile* has been suggested as a cause
- 278 calves, 144 with diarrhoea (7.6% pos by culture, 39.6% pos by toxin) and 134 controls (14.9%/ 20.9%), in 58 of 102 diary farms (Rodriguez *et al*. EID 2006)
- Sampling time/age related to outcome, not Abs

C. difficile in cattle

- Both PCR ribotypes 017 & 027 detected
- 83% of calf isolates ribotype 078
- Convenience sample of beef (53) and veal (7) samples purchased from butchers
- Meat cultured in enrichment broth first, then alcohol shocked
- *C. difficile* isolated from 20% of samples
- One strain appeared to be 027 related (Rodriguez *et al*. EID 2007)

C. difficile in horses

- 1st described in mid 1980s
- Still relatively little known about this problem
- Expensive race horses in USA have died from fulminant colitis
- Most commonly associated with Ab exposure (ery, tmp/ sul, β-lactams, gent)



C. difficile in horses

- Carriage of *C. difficile* by adult horses is rare
- Stress may play a role in disease (possibly for all animals)
- Withholding roughage a risk (VFAs)
- Both foals with & without out diarrhoea get infected (colonised)
- Dams of foals treated with erythromycin for *Rhodococcus equi* get severe disease
- Wide range of ribotypes found in horses

Baverud et al *Equine Vet J* 2003

Group	No.	Culture/toxin +ve
Normal	273	0
Acute colitis+Ab	43	18 (42%)
Colitis, no Ab	72	4 (6%)
No disease+Ab	47	1 (2%)
Colic, no Ab	65	0
	500	23

What does this all mean?

- Approx. 20 years ago no overlap between dog/cat and human strains
- Now most animal isolates produce binary toxin (horses 43%, piglets 83%, cattle 100%)
- Increase in human strains producing binary toxin <1990 0%, 1991-9 24%, 2000-1 45% (Spiggalia *et al*. J Med Microbiol 2004; 53: 1129-36)
- Binary toxin producing strains occurring more in the community, and causing more severe disease

- Pigs & cattle share ribotype 078 – human infections in Europe
- Probably all young animals are colonised with *C. difficile*
- *C. difficile* could contaminate meat during processing and obviously does in North America
- Clostridial spores can be found in meat
  (Vengut *et al.* Equin Vet J 2003; 35: 514-16)
- But is meat consumption a risk?

Table. Source and characteristics of *Clostridium difficile* isolates obtained from retail meats sold in Tuscon, Arizona, USA, 2007*

Meat product	No. samples cultured	Total no. (%) positive	Ribotype	Toxinotype	Δ*tcdC*, bp†	PFGE type	No. (%) pos
Ground beef (uncooked)	26	13 (50)	027	III	18	NAP1	1 (3.8)
						NAP1-related	2 (7.7)
			078	V	39	NAP7	8 (30.8)
						NAP8	2 (7.7)
Summer sausage (ready to eat)	7	1 (14.3)	027	III	18	NAP1	1 (14.3)
Ground pork (uncooked)	7	3 (42.9)	027	III	18	NAP1-related	1 (14.3)
			078	V	39	NAP7	2 (28.6)
Braunschweiger (ready to eat)	16	10 (62.5)	027	III	18	NAP1	2 (12.5)
						NAP1-related	1 (6.2)
			078	V	39	NAP7	7 (43.8)
Chorizo (uncooked)	10	3 (30.0)	027	III	18	NAP1-related	1 (10.0)
			078	V	39	NAP7	2 (20.0)
Pork sausage (uncooked)	13	3 (23.1)	027	III	18	NAP1-related	1 (7.7)
			078	V	39	NAP7	2 (15.4)
Ground turkey (uncooked)	9	4 (44.4)	078	V	39	NAP7	4 (44.4)
Totals	88	37 (42.0)	027	III	18	NAP1	4 (4.4)
						NAP1-related	6 (6.7)
			078	V	39	NAP7	25 (27.8
						NAP8	2 (2.2)

*All samples were positive for *cdtB*, which encodes the binding component of binary toxin. PFGE, pulsed-field gel electrophoresis.
†Deletions in *tcdC* regulatory gene.

Songer *et al. Emerg Infect Dis* 2009; 15: 819-821

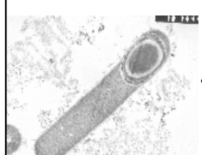
Emergence of *Clostridium difficile* Infection Due to a New Hypervirulent Strain, PCR Ribotype 078

- Compared 027 infections with 078
- 078 increased from 3% to 13% (2005-8)
- In parts of The Netherlands where 90% of pig farms 22.4%
- 078 patients were younger (67 vs 74 yrs)
- More community acquired 17.5% vs 6.7%
- Severity and attributable mortality similar
- Pig & human 078 strains genetically indistinguishable

Goorhuis *et al* Clinical Infectious Diseases 2008; 47:1162–70

Ribotype 078 now the 3rd most common isolate in Europe (Bauer *et al* ECCMID 2009)

Why is this happening?



+ antibiotics = disaster

Table 1. Production of food animals (including export of live animals) and the production of meat and milk, Denmark

DANMAP 2006

Year	Broilers		Turkeys a)		Cattle (slaughtered)		Dairy cows		Pigs		Farmed fish	
											Fresh water	Salt water
	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	1,000 heads	mill. kg	mill. kg	mill. kg
1990	94,560	116	571	2.5	789	219	753	4,542	16,425	1,260	-	-
1992	107,188	137	761	5.4	862	236	712	4,405	18,442	1,442	35	7
1994	116,036	152	1,091	8.6	813	210	700	4,442	20,651	1,604	35	7
1996	107,895	149	961	9.3	789	198	701	4,494	20,424	1,592	32	8
1998	126,063	168	1,124	11.6	732	179	669	4,468	22,738	1,770	32	7
2000	133,987	181	1,042	10.3	691	171	636	4,520	22,414	1,748	32	7
2001	136,603	192	1,038	12.6	653	169	623	4,418	23,199	1,836	31	8
2002	136,350	190	965	11.5	668	169	610	4,455	24,203	1,892	32	8
2003	129,861	181	510	7.4	625	161	596	4,540	24,434	1,898	34	8
2004	130,674	181	55	1.0	632	165	563	4,434	25,141	1,965	34	9
2005	120,498	180	158	0.5	549	145	558	4,449	25,758	1,988	31	8
2006	105,888	163	32	0.1	509	140	563	4,492	25,763	1,957	-	-

Data from Statistics Denmark (www.dst.dk) and The Danish Directorate for Fisheries
a) From 2002, the export of live turkeys for slaughter increased. By 2004, 95% of all turkeys raised in Denmark were slaughtered abroad. For turkeys, data on export of live animals is not included in the table

Approx. 50% increase in numbers

Table 4. Trends in the estimated total consumption (kg active compound) of prescribed antimicrobials for production animals, Denmark

DANMAP 2006

ATC_{vet} group b)	Therapeutic group	1990	1992	1994	1996	1998	2000	2001	2002	2003	2004	2005	2006
QJ01AA	Tetracyclines	9,300 a)	22,000	36,500	12,900	12,100	24,000	28,500	24,500	27,300	29,500	30,050	32,650
QJ01CE	Penicillins, β–lactamase sensitive	5,000	6,700	9,400	7,200	14,300	15,100	16,400	17,400	19,000	20,900	22,250	22,600
QJ01C/QJ01DA	Other penicillins, cephalosporins	1,200	2,500	4,400	5,800	6,700	7,300	8,800	9,900	11,100	12,900	12,300	11,550
QJ01EW	Sulfonamides + trimethoprim	3,800	7,900	9,500	4,800	7,700	7,000	9,200	10,600	10,600	11,500	12,200	13,800
QJ01EQ	Sulfonamides	8,700	5,900	5,600	2,100	1,000	1,000	950	900	850	850	750	750
QJ01F/QJ01XX	Macrolides, lincosamides, pleuromutilins	10,900	12,900	11,400	7,600	7,100	15,600	18,400	19,200	20,700	24,200	22,350	22,050
QJ01G/QA07AA	Aminoglycosides	7,700	8,500	8,600	7,100	7,800	10,400	11,600	11,700	11,700	11,600	10,800	10,500
	Others c)	6,700	6,800	4,400	600	650	300	900	1,600	1,500	1,000	1,950	1,250
Total		53,400	73,200	89,900	48,000	57,300	80,700	94,700	95,900	102,500	112,500	112,650	115,150

1990-2000: Data based on reports from the pharmaceutical industry of total annual sales. (Data 1990-1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). 1996-2000: Danish Medicines Agency). Data 2001-2006: VetStat. For comparability between VetStat data and previous data, see DANMAP 2000. Only veterinary drugs are included. Veterinary drugs almost exclusively used in pets (tablets, capsules, ointment, eye/ear drops) are excluded. Dermal spray with tetracycline, used in production animals, is the only topical drug included
a) Kg active compound rounded to nearest 50 or 100
b) Only the major contributing ATC_{vet} groups are mentioned
c) Consumption in aquaculture was not included before 2001

Approx. 400% increase in penicillins, β-lactamase sens.

Other penicillins, cephalosporins 1000% increase

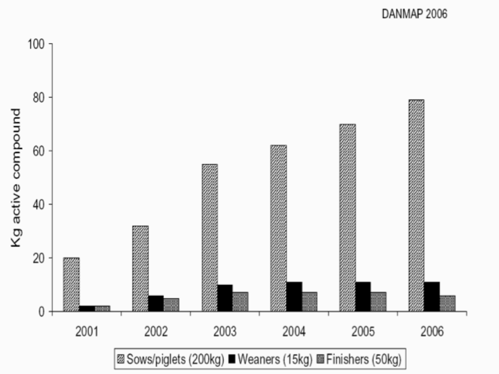


Figure 4 . Consumption of cephalosporins in pigs given as kg active compound from 2001 to 2006, Denmark

DanMap 2007

"This change in prescription habits suggests that the consumption of cephalosporins in pigs is changing from occasional prescription to more systematic prescription in herds producing 14-29% of the weaned pigs."

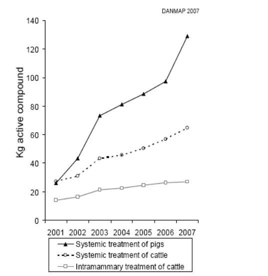


Figure 7. Use of 3rd and 4th generation cephalosporins in pigs and cattle, 2001-2007, Denmark

Population per km²

- 20 South Korea 498
- 24 The Netherlands 395
- 73 People's Republic of China 138
- 81 Denmark 126
- 177 United States of America 31
- 232 Australia 2.84

What are the risks from animal exposure?

- Direct exposure to pigs, horses, cattle colonised/infected with *C. difficile* and associated dust contaminated with *C. difficile* spores in piggeries, stables.
- Direct exposure to waste/compost made from pig litter and contaminated with *C. difficile* spores.
- Exposure through the handling or consumption of contaminated meat and meat products.

How can this be fixed?

- Stop giving animals broad-spectrum cephalosporins
- Stop giving humans broad-spectrum fluoroqinolones
- Improved cleaning practices in healthcare institutions
- ???????? Improved cleaning practices in production animal industries

Infection control (Baverud *Vet Clin Equine* 2004)

- Restrict use of antimicrobials – lincosamides, macrolides & cephalosporins
- Minimise stress factors
- Reduce environmental contamination (How?)
- Isolate animals with diarrhoea
- Avoid accidental ingestion of erythromycin by dams
- Hand hygiene & gloves
- Test suspect animals


Bojesen AM, Olsen KE, Bertelsen MF. Fatal enterocolitis in Asian elephants (Elephas maximus) caused by Clostridium difficile. Vet Microbiol 2006 10;116:329-35.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