

WGS and antimicrobial resistance

Isabelle Kempf

ANSES Ploufragan Laboratory, Mycoplasmaology, Bacteriology and Antimicrobial
Resistance Unit

Antimicrobial Resistance NRL



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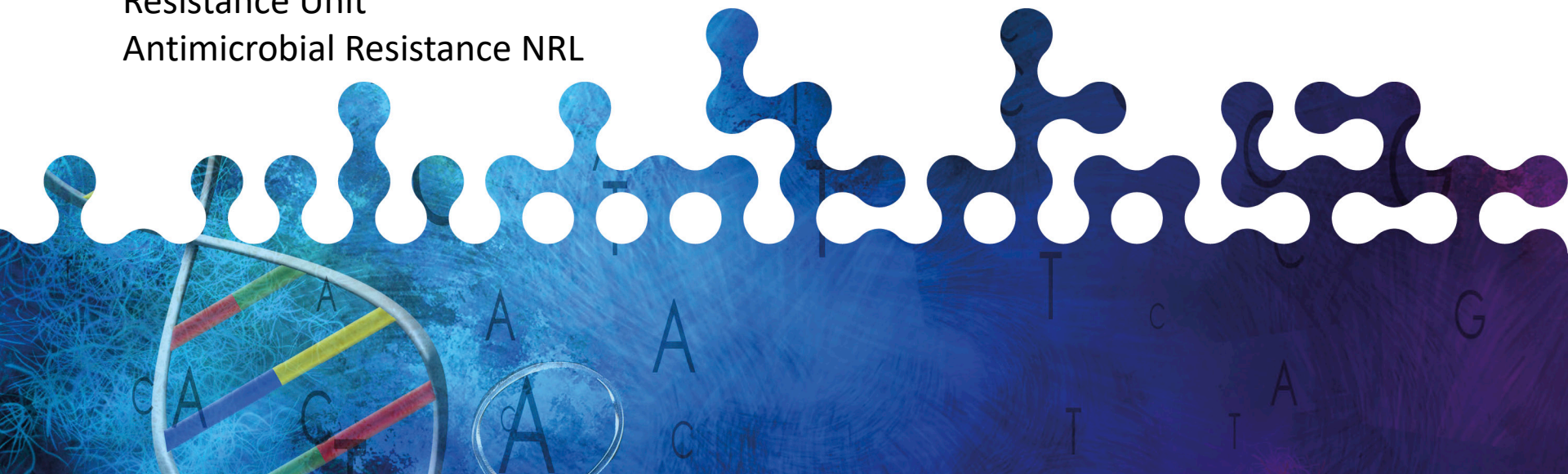
How useful is WGS?

(Can non-bioinformaticians use it?)

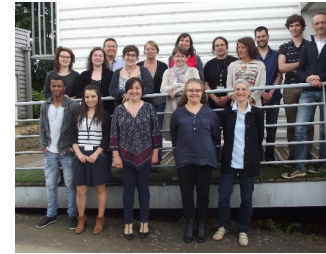
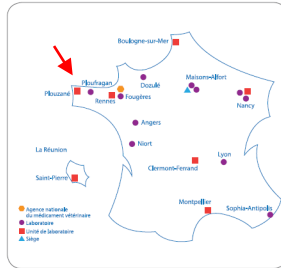
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Antimicrobial Resistance NRL



Anses Ploufragan, MBA Unit: our activities on antimicrobial resistance



- **Monitoring AMR in poultry, pigs, fish and their environment**
 - Zoonotic bacteria (*Campylobacter*, Decision 2013/652/EU)
 - Commensal bacteria: *E. coli* from healthy animals
 - Pathogenic bacteria: Resapath network (*E. coli*) and Mycoplasmas
- **Impact of agricultural or veterinary practices**
 - Antimicrobial administration, manure treatments...
 - Impact on selection of resistant bacteria, on the microbiote, on the resistome
- **Alternatives to antimicrobials**
 - Probiotics, bacteriocins, vaccines...

Resistance to extended-spectrum-cephalosporins (ESC) in *Enterobacterales*

- ESC: critically important antibiotics for human health
- ESC-Resistance (ESCR): Increased incidence in humans since 2000s
- ESC-Resistance present also in bacteria of animal origin
- Resistance genes are mostly borne by conjugative plasmids and encode Extended Spectrum Beta-Lactamases (ESBL) or AmpC beta-lactamases



Resistance to ESC: Questions



- Diversity of the resistance mechanisms and their genetic supports, in commensal and pathogenic bacteria?



Characterization of isolates

- Origin, diffusion, persistence of resistance genes in animals, in farms, in the environment? → Longitudinal study in free-range broilers
- Diffusion or loss of resistance genes in animals? → Experimental studies in inoculated animals



Characterization of poultry ESCR isolates

- *E. coli* isolates resistant to ESC in poultry
 - In 2010-2012: peak of the ESC resistance
 - Diversity? Resistance genes? Virulence genes?
- Focus on 31 *bla*_{CTX-M-1} isolates, most prevalent
 - Commensal (14) and pathogenic *E. coli* (17)
 - Various types of chickens (conventional, organic, free range)
 - Various geographic areas from France
 - Various hatcheries, farms, slaughterhouses





Characterization of poultry ESCR isolates

• Results 1/3: Conventional methods

30/31 PFGE profiles, Phylo. Groups, MICs

strain	Gallus Type ^a	Hatchery code	Farm ^b	Slaughterhouse ^b	Age ^c	Phylog. Gp	TET	SMX	TMP	STR	CIP
COV2	O	6	C	C	90	F					
COV3		3	E	L	NR	D					
COV4		6	I	F	33	E					
COV5	E	2	J	K	32	E					
COV6		5	H	B	37	A					
COV7		1	A	B	NR	C					
COV10		7	G	I	37	B1					
COV11	E	2	K	K	34	B1					
COV12	E	2	K	K	33	A					
COV13		5	H	I	38	A					
COV14		4	L	L	53	A					
COV15		5	B	B	37	A					
COV16		3	A	B	39	F					
COV17						C					
COV18						A					
COV19						C					
COV20						F					
COV21						F					
COV22						F					
COV23						E					
COV24						C					
COV25						E					
COV26						C					
COV27						F					
COV28						F					
COV29						C					
COV30						B2					
COV31						B2					
COV32						F					
COV33						F					

Micro-array: virulence genes (Anjum et al.)

strain	ABC transporter protein	adherence protein	Cytolysin distending toxin B	Enterotoxin siderophore receptor protein	Increased serum survival	long polar fimbriae	MchC protein	Microcin H47	P. related fimbriae	Siderophore receptor	temp sensitive hemagglutinin	vacuolating autotransporter protein	SPATE
COV1													
COV9													
COV4													
COV5													
COV6													
COV8													
COV10													
COV12													
COV13													
COV14													
COV15													
COV17													
COV18													
COV20													
COV21													
COV22													
COV23													
COV25													
COV26													
COV27													
COV29													
COV30													
COV32													
COV33													

Fecal
 Colibacillosis

Important strain diversity?

Characterization of poultry ESCR isolates



• Results 3/3: Comparison of sequences

Comparison of the 30 *bla*_{CTX-M-1} plasmids for the 114,313 common nt (but alignment could contain gaps), showed that overall they shared 67% identity.

- Two plasmids from distinct commensal isolates showed **100% identity**

strain	Gallus Type ^a	Hatch ery code	Farm ^b	Slaug herho ^b use	Age ^c	Phylo g. Gp	TET	SMX	TMP	STR	CIP
COV2	O	6	C	C	90	E					
COV7		1	A	B	NR	C					

- One plasmid from a commensal strain and seven plasmids from colibacillosis shared **more than 99% identity** although the susceptibility/phylogenetic groups/virulence profile of their parental strains were different

Diversity of strains but low diversity for ARG and plasmids in broilers isolates


Main results for ESCR pig isolates



- Mainly carried by highly **similar *bla*_{CTX-M-1} IncI1/ST3 plasmids**.
- These plasmids very often bear *sul2*, *dfrA17* and *aadA5*, and occasionally *tet(A)*, *mph(A)*, *erm*, *floR* or *strA*, *strB*. Resistance genes for quinolones or polymyxins were never detected.
- Few virulence genes (including colicins, heat-stable enterotoxins, adhesins or temperature-sensitive hemagglutinins).
- Occasionally: *bla*_{CTX-M-27} and *bla*_{CTX-M-14}, the latter being on an IncF plasmid which showed very close identity to a human epidemic plasmid.

Resistance to ESC: Questions



- Diversity of the resistance mechanisms and their genetic supports, in commensal vs pathogenic bacteria? → Characterization of isolates
- Origin, diffusion, persistence of resistance genes/plasmids in animals, in farms, in the environment?
-  **Longitudinal study in free-range broilers**
- Diffusion or loss of resistance genes in animals? → Experimental studies in inoculated animals

Longitudinal study in free-range broilers



- Two hatcheries, 11 flocks, 9 farms
- Fecal samples collected in farms from D0 (before arrival) to D77
- Isolation of ESCR *E. coli*: characterization of isolates; plasmids sequenced from transformants or transconjugants

Longitudinal study in free-range broilers



Results 1/4: *bla*_{CTX-M-1} *Incl1* pMLST3 plasmids

15 *E. coli* (13 PFGE profiles) with *bla*_{CTX-M-1} *Incl1* pMLST3 plasmids obtained from **D0 to D77** from 7 flocks on 6 farms

Most contained the *tet(A)* and *sul2* genes.

Seven plasmids from isolates of different phylogenetic groups obtained from Day 2 to Day 41, from 5 flocks, 2 hatcheries shared sequence **identity higher than 99%**.

Longitudinal study in free-range broilers



Results 2/4: *bla*_{CTX-M-1} *Incl1* pMLST3 plasmids

But the *bla*_{CTX-M-1} *Incl1* pMLST3 sequences were also very close (96% coverage, 99% identity) to:

- *E. coli* pC49-108 obtained from a chicken in Switzerland
- *E. coli* FAP1 from pig faeces in the Netherland
- *E. coli* pTC_N40607 obtained from retail meat and cattle farms in the US...

Longitudinal study in free-range broilers



Results 3/4: bla_{CMY-2} isolates

10 isolates obtained from D0 to D7 with **bla_{CMY-2}** , *all from the same hatchery.*

Six *E. coli* isolated from Day 0 to Day 7 from three flocks on F13 and F14, from chicks that had been hatched on the same day in the same hatchery were CTX-FOX-TMP-SMX-TET-CIP-CHL-resistant.

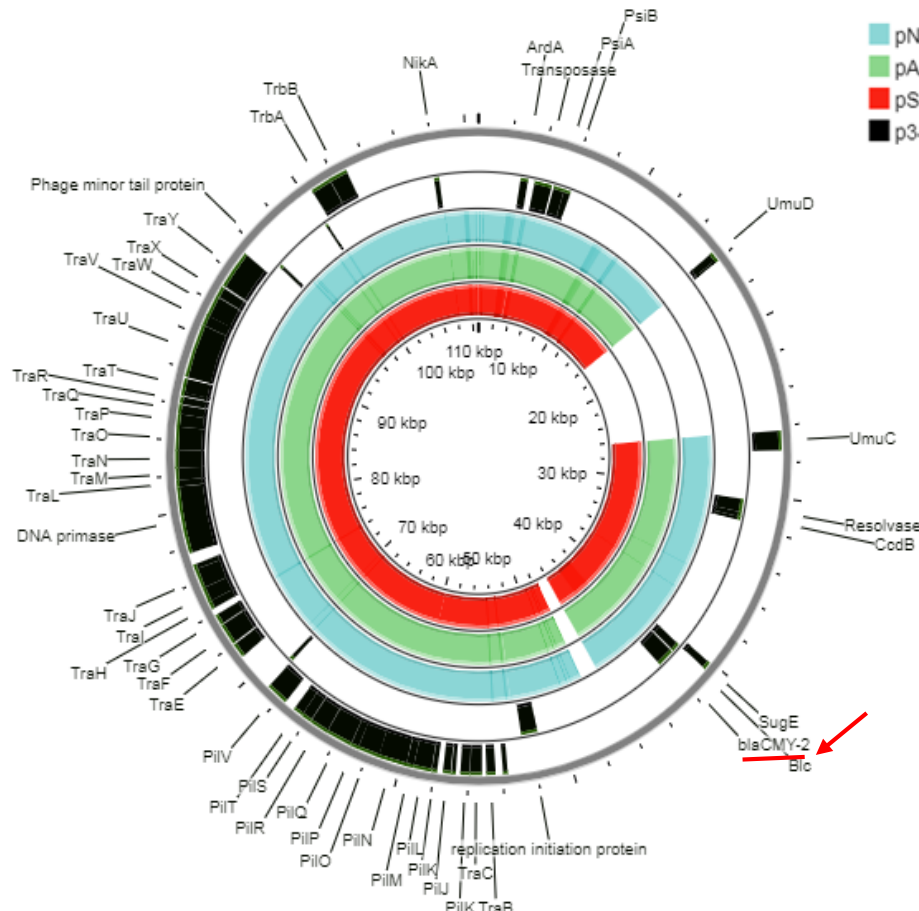
Longitudinal study in free-range broilers



Results 4/4: *bla*_{CMY-2} plasmids

- 10 *bla*_{CMY-2} IncI1 pMLST12, 110-117 kbp
- No resistance genes other than *bla*_{CMY-2}, no virulence genes
- Very similar to each other (cover.98-100%, ident. 99-100%)
- And very similar to *E. coli* M63 plasmid from a pig in France (coverage 99%, identity 100%), but lacked a 13 kbp region (*aadA5*, *sul2* *dfrA17*, *IntI1*, IS26, and mobile elements)
- And also very similar
 - pN13-01290 from *S. Heidelberg* from turkey meat in Quebec
 - pAMR588-04-00320 from *S. Heidelberg* from chicken faeces in Ontario
 - pSTY1-2010K-1587 from *S. Typhimurium* from human stool in the USA

Longitudinal study in free-range broilers

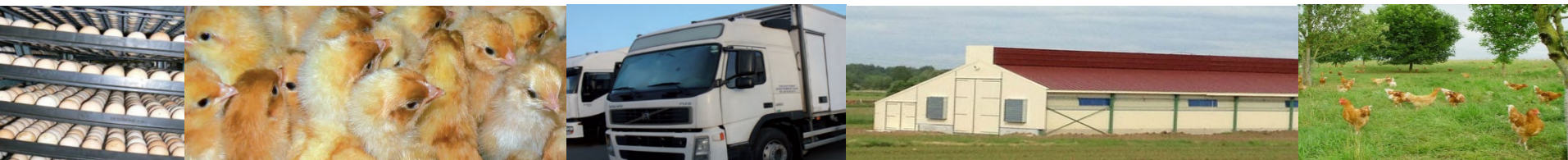


- pN13-01290
- pAMR588
- pSTY1
- p343-2

S. Heidelberg /turkey meat /Quebec
 S. Heidelberg / chicken faeces / Ontario
 S. Typhimurium / human stool /USA
 This study *E. coli* / broilers / France

***bla*_{CMY-2} IncI1 pMLST12**

Longitudinal study in free-range broilers: main results



- The IncI1 pMLST3 *bla*_{CTX-M-1} plasmids were present in isolates obtained from Day 0 to Day 77 from both hatcheries, and were very similar both to each other and to plasmids very frequently described in French broilers, and in other animal species and regions. Their epidemic nature therefore makes it difficult to determine the origin of contamination.
- On the contrary, the IncI1 pMLST12 *bla*_{CMY-2} plasmids are uncommon in French isolates from poultry. In this study, they were only obtained from the isolates of young birds from hatchery B, were very similar to each other, suggesting a probable vertical transmission.

Resistance to ESC: Questions



- Diversity of the resistance mechanisms and their genetic supports, in commensal vs pathogenic bacteria from different animal species and humans? Characterization of isolates
- Origin, diffusion, persistence of resistance genes/plasmids in animals, in farms, in the environment?
- Diffusion or loss of resistance genes in animals?



Experimental studies in inoculated animals

In vivo loss of multiresistance plasmid

- 4 different trials in controlled animal facilities, 51 SPF pigs
- Inoculated or contact pigs
- *E. coli* M63, rifampicin resistant; contains pESCR: IncI1 pMLST12 with *bla*_{CTX-M-1}, *bla*_{CMY-2}, *sul2*, *dfrA17*, and *aadA5* genes

 TABLE 1 Design of animal experiments and culture results^c

Trial	Group receiving M63 inoculation on day 0 (dose per pig)	Other inoculation(s) (dose per pig)	Fecal sample origin(s)	No. of samples	No. of rifampin-resistant <i>E. coli</i> isolates	No. of CTX-susceptible <i>E. coli</i> isolates/total no. of isolates
04-12	Inoculated pigs (6.8 × 10 ¹⁰ CFU ^a)	Reinoculation with M63 on day 1	6 inoculated pigs sampled on days 1 to 11 and days 14, 16, 18, 20, 22, ^b 24, ^b and 27 ^b	97	97	0/97
	Contact pigs (no inoculation)		4 contact pigs sampled on days 1 to 11 and days 14, 18, 16, 20, 22, ^b 24, ^b and 27 ^b	66	55	2/55
10-12	All pigs (78 × 10 ⁹ CFU)	ED1a (days 8–12) (11.2 × 10 ⁹ CFU)	5 pigs sampled on days 2, 3, ^b 9, and 13 ^b	16	15	0/15
		EcN (days 8–12) (17 × 10 ⁹ CFU)	5 pigs sampled on days 2, 3, ^b 9, ^b 13, ^b and 16 ^b	14	13	0/13
01-13	All pigs (78 × 10 ⁸ CFU)	ED1a (days 8–13) (4.4 × 10 ⁹ CFU)	8 pigs sampled on days 1, 3, 7, 10, 13, 17, and 22	55	45	0/45
06-13	All pigs (73 × 10 ⁶ CFU)	No	8 pigs sampled on days 3, 10 ^b , 13, 16, 20, 23, 27, and 31 ^b	55	51	1/51
		ED1a (days 8–13) (74 × 10 ⁶ CFU)	7 pigs sampled on days 3, 10, ^b 16, 20, 23, ^b 27, and 31 ^b	42	42	0/42
		ED1a (days 8–13) (65 × 10 ⁷ CFU)	8 pigs sampled on days 3, 16, ^b 20, ^b 23, ^b 27, ^b and 31 ^b	36	36	0/36

^a Pigs were inoculated on day 0 and day 1.

^b Not all pigs were sampled.

^c There were 48 pigs inoculated with M63 that were not yet or not inoculated with ED1a or EcN with 200 samples, with 1 being a CTX-susceptible *E. coli* isolate out of 194 Rif^r *E. coli* isolates; there were 4 contact pigs with 66 samples, with 2 being CTX-susceptible *E. coli* out of 55 Rif^r *E. coli* isolates; there were 28 pigs inoculated with M63 and then with ED1a with 149 samples, with 0 being CTX-susceptible *E. coli* out of 99 Rif^r *E. coli* isolates; and there were 4 pigs inoculated with M63 and then with EcN with 6 samples, with 0 being CTX-susceptible *E. coli* out of 5 Rif^r *E. coli* isolates. In total, there were 3 CTX-susceptible *E. coli* isolates out of 353 Rif^r *E. coli* isolates.

In vivo loss of multiresistance plasmid

- **Results**
- 84% of 355 fecal samples from inoculated pigs and 83% of 66 from contact pigs contained *E. coli* M63
- Analysis of 353 Rif-R *E. coli*
 - 350 positive by PCR for *bla*_{CTX-M-1}, *sul2*, *Incl1*
 - 3 negative for *bla*_{CTX-M-1}, *sul2*, *Incl1*, no growth on CTX → WGS

In vivo loss of multiresistance plasmid

- pESCR covered 72 times on average for *E. coli* M63
- Average coverage depth of 1 for the three Rif-R CTX-S isolates
- *E. coli* M63 and the three Rif-R CTX-S isolates share the same chromosomal sequences

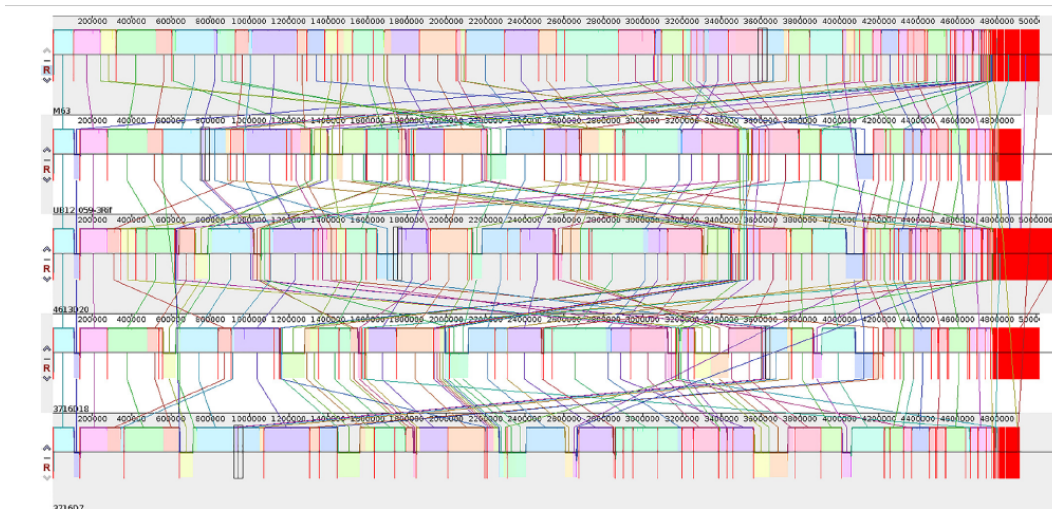


FIG 1 Chromosome assemblies of *E. coli* M63, UB12/059-3Rif, and the three rifampin-resistant cefotaxime-susceptible *E. coli* isolates reordered according to M63 with Mauve, aligned by using progressiveMauve. M63 is the inoculated strain; UB12/059-3Rif is the recipient strain used to prepare *E. coli* M63; and 3716D7, 3716D18, and 4613D20 are the rifampin-resistant cefotaxime-susceptible *E. coli* isolates obtained from pig 3716 on days 7 and 18 and pig 4613 on day 20, respectively.

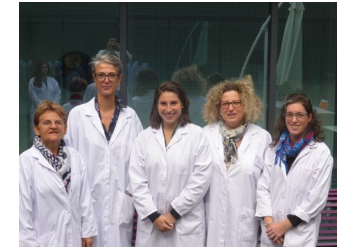
Conclusion

WGS is helpful for the study of:

- Diversity of resistant isolates, resistance genes, involved plasmids... in animals, humans and the environment
- Detection of epidemic clones, plasmids
- Presence of different ARG (co-selection) or virulence genes on the same genetic support
- Origin, persistence, diffusion, loss of resistance, *in vivo* at different scales (animal, flock, population)

Thank you to

- Mycoplasmaology, Bacteriology and Antimicrobial Resistance Unit (S. Baron, C. de Boisséson, E. Jouy, E. Larvor, L. Le Devendec, G. Mourand)
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Reducing antibiotic use in veterinary medicine



Thank you for your attention