Table 1 Antimicrobial resistance in enterobacteria from the faeces of wild moose, deer and vole

Bacteria (no. of isolates)	Percentage resistant (MIC_{50}; MIC_{90})						
	AMP	*AMC*	*CEX*	*CXM*	*CTX*	*ATM*	*IPM*
	⩾32*	⩾32/16	⩾32	⩾32	⩾64	⩾32	⩾16
Escherichia coli (98)	0 (4; 8)	0 (4; 4)	0 (8; 8)	0 (4; 4)	0 (0.06; 0.06)	0 (⩽0.06; 0.1)	0 (⩽0.2; ⩽0.2)
Enterobacter agglomerans group (48)	NA (8; 16)	NA (4; 4)	NA (8; 16)	4 (4; 4)	0 (0.06; 0.1)	0 (⩽0.06; 0.1)	0 (0.5; 1)
Yersinia spp. (29)	NA (32; 64)	NA (32; >64)	NA (>64; >64)	7 (4; 4)	0 (0.2; 0.5)	0 (0.5; 0.5)	0 (0.5; 1)
Serratia spp. (11)	NA (32; 128)	NA (8; >64)	NA (>64; >64)	82 (64; >64)	0 (0.5; 1)	0 (0.5; 0.5)	0 (0.5; 0.5)

Bacteria (no. of isolates)	Percentage resistant (MIC_{50}; MIC_{90})							
	GEN ⩾16	*STR* (⩾32)	*NAL* ⩾32	*CIP* ⩾4	*CHL* ⩾32	*TET* ⩾16	*TMP* ⩾16	*SUL* ⩾512
Escherichia coli (98)	0 (0.5; 0.5)	1 (4; 4)	0 (2; 4)	0 (0.03; 0.06)	0 (4; 8)	0 (1; 2)	0 (0.2;0.5)	0 (16;32)
Enterobacter agglomerans group (48)	0 (0.2; 0.2)	0 (2; 2)	0 (1; 4)	0 (0.03; 0.1)	0 (⩽2; ⩽2)	0 (1; 1)	0 (⩽0.06; 0.1)	0 (8; 16)
Yersinia spp. (29)	0 (0.2; 0.5)	0 (2; 4)	0 (1; 1)	0 (0.01; 0.03)	0 (8; 8)	0 (2; 2)	0 (1; 2)	0 (16; 64)
Serratia spp. (11)	0 (0.2; 0.5)	0 (2; 4)	0 (2; 2)	0 (0.06; 0.1)	0 (4; 16)	NA (2; 64)	0 (0.2; 0.5)	0 (16; 32)

MIC_{50} and MIC_{90} are the antibiotic concentrations (mg l^{-1}) at which 50 and 90%, respectively, of the tested population is inhibited from growing. AMP, ampicillin; AMC, amoxycillin/clavulanic acid; CEX, cephalothin; CXM, cefuroxime; CTX, cefotaxime; ATM, aztreonam; IPM, imipenem; GEN, gentamicin; STR, streptomycin; NAL, nalidixic acid; CIP, ciprofloxacin; CHL, chloramphenicol; TET, tetracycline; TMP, trimethoprim; SUL, sulphamethoxazole. NA, not applicable: most strains or species intrinsically resistant.
*Resistance breakpoint (mg l^{-1}) according to the National Committee for Clinical Laboratory Standards.

tions of the same bacterial species that have or have not been exposed to humans.

Fresh faeces from newly felled moose (*Alces alces*; $n=16$) and white-tailed deer (*Odocoileus virginianus*; $n=7$) were collected in the autumn of 1999 by hunters in two areas of Uusimaa, southern Finland. Faecal pellets were prepared from bank voles (*Clethrionomys glareolus*, $n=23$) trapped in Ostrobothnia, western Finland[4]; these had been stored whole at −20° C for less than one year. Five bacterial colonies per sample, representing all different colonial morphologies present, were identified to at least genus level, and the minimum inhibitory concentrations (MICs) of 15 antibiotics were determined, as previously described[5].

The ungulate faecal flora was similar to human flora, with *Escherichia coli* as the main species. In vole faeces, *Enterobacter agglomerans* and *Yersinia* spp. dominated. Results are given only for genera represented by more than four isolates. The only resistance found was to cefuroxime (Table 1) and to streptomycin (in one sample of *E. coli*; this could be transferred by conjugation[6] to *E. coli* C600). Most of the cefuroxime resistance was, as judged from MIC profiles, most likely caused by a class A (Bush group 2e) cefuroximase similar to the chromosomal *Proteus vulgaris* enzyme[7], and was thus most probably indigenous. It was found not to be caused by the most common transferable class-A β-lactamases: the cerufoxime-resistant strains were tested by using the polymerase chain reaction for the presence of TEM and SHV[8], but only one strain contained a TEM-type enzyme and none carried SHV.

These results disagree with those from a study of enterobacteria from wild English rodents, where extremely high resistance was found[1]. The English study questions the usefulness of restricting antibiotic use, as these rodents are presumed to have had no contact with antibiotics. However, the overall load from antibiotic use in England is larger than in Finland: the mean number of inhabitants per square kilometre in Finland is 17, compared to 378 in England (see www.statistics.gov.uk and www.stat.fi); also, the load from agriculture is less in Finland — there are, for example, ten times fewer cattle and five times fewer pigs than in the UK (see www.stat.fi and www.maff.gov.uk). Since 1996, the use of antibiotic additives in animal feed has gradually been abandoned, but occasional contact cannot be ruled out. Our sampled populations almost certainly represent wild animal populations better.

In faecal flora, *E. coli* is the species showing the most resistance (resistance to streptomycin, to sulphamethoxazole, and to tetracycline is highest at 14–18%, even in healthy people)[5]. Resistance is known to increase with increased exposure to antibiotics and during hospitalization[5]. Our finding of an *E. coli* population that has never been exposed to humans and which is free of resistance to antibiotics strongly suggests that the widespread resistance found in all *E. coli* populations associated with humans must be caused by human activities. Antibiotic restrictions whenever feasible are still very much on the agenda.

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Gilliver et al. *reply* — The study by Österblad *et al.* confirms the importance of understanding the role of commensal bacteria, particularly in wildlife, in the ecology of antibiotic resistance. The two studies combined suggest that the gut flora of wildlife populations with very little or no contact with either humans or anthropogenic antibiotics (Österblad *et al.*'s study) may have negligible levels of antibiotic resistance, whereas wildlife populations living in closer proximity to humans but still with no known direct contact with anthropogenic antibiotics (our study) may have much higher levels of antibiotic resistance.

These conclusions fit with earlier findings of a higher prevalence of antibiotic resistance among baboons living close to humans than in baboons in more isolated populations[1]. Questions that still need to be addressed concern the extent and frequency of antibiotic exposure necessary to generate significant resistance, what determines the dynamics of decline in resistance following restrictions in antibiotic use, and the nature and extent of any reservoir of antibiotic resistance that may exist in natural environments and which could undermine future attempts to manage resistance.

These questions can only be resolved by thorough spatial and temporal mapping of antibiotic resistance in natural environments. We inferred from our study that it would be unwise to assume that resistance would decline significantly as a consequence of restricted use of antibiotics. This suggestion still holds, because resistance has been maintained for over three years at our study site, over several generations of rodents, without any obvious exposure to antibiotics.

We agree with Österblad *et al.* that antibiotic restrictions should still be very much on the agenda, but that agenda must include concerted attempts to understand what the consequences of restrictions are likely to be.

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