guidelines. Moreover, many horse wounds heal by supportive therapy without antibiotics. Finally, the hypothesis for MRSA being selected by the use of cefquinome in those horses cannot be excluded.

In this study, two CCs with no geographical clustering were characterized. Indeed, three MRSA strains belonged to CC130, the most prevalent CC among *mecC*-positive MRSA in animals, whereas the fourth one belonged to ST49, which was recently described in *mecC*-positive humans. Among the CC130 isolates, two different STs (ST1245 and ST130) and three divergent—though related—*spa* types were found. This finding might be due either to microevolutions of a common ancestor or to successive introduction of distinct strains.

Considering the nosocomial and zoonotic potential of MRSA isolated from horses, equine veterinarians and riders should pay specific attention to hygiene measures in both veterinary clinics and equestrian centres in order to limit MRSA spread. Prudent and responsible use of antibiotics by veterinarians is also of major importance to avoid selection and dissemination of such resistant strains.

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Transparency declarations

None to declare.

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Clonal transmission of a colistin-resistant *Escherichia coli* from a domesticated pig to a human in Laos

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Sir,

Colistin, recently reintroduced in human medicine, is one of the most important antibiotics currently used to treat severe Gram-negative bacterial infections in humans. Unfortunately, it is also extensively used in animal production, including in swine and poultry farming against Gram-negative bacterial pathogens.[1,2]

However, the extensive use of antibiotics in food-animal production has been shown to increase the risk of transferring resistant bacteria to humans.[3] In this study, we investigated the possible link between colistin-resistant *Escherichia coli* isolated from domesticated pigs and humans in a rural area in Laos.

In 2012, faecal samples were collected from 190 healthy individuals and 62 domesticated animals (44 free-range goats and 18 semi-free-range pigs) in Laksip, Laos (GPS coordinates of latitude: 19°50′55.615″N; longitude: 102°10′4.266″E). Approval was obtained from the Ministry of Health Council of Medical Sciences, National Ethics Committee for Health Research Laos, number 51/NECHR. The samples were screened for colistin-resistant *E. coli* using Cepacia Medium (Becton Dickinson, Heidelberg, Germany) and isolates identified using MALDI-TOF MS. Colistin and polymyxin B MICs were determined by Etest on Mueller–Hinton II agar (bioMérieux, France) and microdilution with cation-adjusted Mueller–Hinton II broth (Becton Dickinson, Le Pont de Claix, France) and interpreted using EUCAST[4] and CLSI 2014 guidelines (M100-S24), respectively. Conjugation experiments were conducted as previously described using kanamycin.[5] MLST was performed as described at http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/documents/primersColi_html. Virulence typing was performed by targeting 10 virulence genes (*kpsMTII*, *papC*, *sfa/foc*, *afa/dra*, *iutA*, *iss*, *tsh*, *sitAP*, *ompT* and *hlyF*) and isolates classified as extraintestinal pathogenic *E. coli* (ExPEC) according to the criteria of Johnson *et al*.[6] PFGE typing was performed as described previously[7] using XbaI (Invitrogen, France) and 1.2% PFGE agarose gel (Sigma). Lastly, PCR and sequencing of the *pmrAB*, *phoPQ* and *mgrB* genes of colistin-resistant *E. coli* were carried out.[8]

Six colistin-resistant *E. coli* were isolated from humans, four from pigs and none from goats (Table 1). All of the isolates displayed resistance to amoxicillin and trimethoprim/sulfamethoxazole (Table 1). We did not isolate an *E. coli* strain that was only resistant to colistin, which may be due to the medium's composition.

All six of the *E. coli* isolated from humans and two from pigs belonged to novel STs (Table 1). The remaining colistin-resistant *E. coli* from pigs, ST93 and ST117 (an emerging avian pathogenic *E. coli*), were both known zoonotic pathogenic strains. Interestingly, two colistin-resistant *E. coli*—one from a human (LH1, from a 15-year-old boy, the only household member screened) and one from a pig (P10)—both belonged to the same novel ST and displayed the same virulence and PFGE patterns (Figure S1, available as Supplementary data at *JAC* Online). Further investigation showed that the pig belonged to the boy's family and the boy (with no recent history of antibiotic usage) normally feeds the pig without wearing any protective equipment such as boots. This observation indicates a possible horizontal transmission from pig to human. The colistin-resistant *E. coli* LH1 (from the human) had additional resistance to aminoglycosides and intermediate resistance to ciprofloxacin and was positive for *aadA22* and *aac(6′)-Ib-cr*. *E. coli* LH1 produced a transconjugant resistant to kanamycin, indicating that it has further acquired a mobile element bearing aminoglycoside resistance genes. Of these 10 colistin-resistant *E. coli* isolates, 4 (3 from humans and 1 from a pig) were classified as potential ExPEC (Figure S1).

Lastly, no known mutations associated with colistin resistance were observed in *pmrAB*, *phoPQ* and *mgrB* from eight of the isolates, which could indicate the participation of other unknown gene(s) in polymyxin resistance in *E. coli*. However, two of the *E. coli* (LH57 and LH140) had a unique missense mutation of E375K in PhoQ. *In silico* analysis using PROVEAN software (http://provean.jcvi.org/seq_submit.php) predicted the mutation as deleterious and it may thus contribute to resistance by affecting the activity of PhoQ. Moreover, mutations in PmrAB, another two-component system, have been linked to colistin resistance in *E. coli* from animals.[8] Daily subculturing of three colistin-resistant *E. coli* (LH1, LH57 and P10) in colistin-free Mueller–Hinton medium up to 30 passages resulted in one (P10) colistin-susceptible revertant (6 to 0.094 mg/L) at the 18th passage, indicating that colistin resistance is reversible in some *E. coli* strains and more stable in others.

Polymyxins, mostly in combination with other antibiotics in the form of premixes, are heavily used by farmers in South-East Asia.[2,9] The unregulated use of polymyxins in animal farming could significantly contribute to the emergence of colistin resistance among zoonotic bacterial pathogens and such pathogens could find their way to humans. This is because antimicrobial drug use is regarded as the most powerful factor contributing to the emergence, selection and spread of antibiotic-resistant microorganisms and genes in animals and humans.[10] As an

Table 1. Antibiotic resistance patterns and STs of colistin-resistant *E. coli* isolated from humans and pigs

	E. coli isolate (ST)									
	humans						pigs			
Antibiotic	LH30 (ST4012[a])	LH57 (ST3997[a])	LH1 (ST4015[a])	LH121 (ST4013[a])	LH140 (ST3997[a])	LH257 (ST4014[a])	P10 (ST4015[a])	P6 (ST4704[a])	P17 (ST93)	P7 (ST117)
PMB (mg/L)[b]	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)
CST (mg/L)[b]	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)	R (4)
CST (mg/L)[c]	R (6)	R (8)	R (6)	R (16)	R (12)	R (12)	R (6)	R (6)	R (4)	R (4)
AMX	R	R	R	R	R	R	R	R	R	R
AMC	S	S	I	S	S	S	I	I	S	S
CRO	S	S	S	S	S	S	S	S	S	S
CTX	S	S	S	S	S	S	S	S	S	S
ATM	S	S	S	S	S	S	S	S	S	S
FOX	S	S	S	S	S	S	S	S	S	S
TIM	S	S	S	S	S	S	S	S	S	S
IPM	S	S	S	S	S	S	S	S	S	S
TOB	R	R	R	S	R	S	S	S	S	R
GEN	R	R	R	S	R	S	S	S	S	R
AMK	I	R	S	S	I	S	S	S	S	S
KAN	R	R	R	S	S	S	S	S	S	S
CIP	R	R	I	I	R	S	S	S	S	S
OFX	R	R	S	S	R	S	S	S	S	S
SXT	R	R	R	R	R	R	R	R	R	R

PMB, polymyxin B; CST, colistin; AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; CRO, ceftriaxone; CTX, cefotaxime; ATM, aztreonam; FOX, cefoxitin; TIM, ticarcillin/clavulanic acid; IPM, imipenem; TOB, tobramycin; GEN, gentamicin; AMK, amikacin; KAN, kanamycin; CIP, ciprofloxacin; OFX, ofloxacin; SXT, trimethoprim/sulfamethoxazole; R, resistant; S, susceptible; I, intermediate.
[a]Novel ST.
[b]Broth microdilution method.
[c]Etest.

immediate priority, the use of the same classes of antibiotics used in human medicine, such as colistin, in animal production, should be restricted. Moreover, colistin has been reclassified as 'critically important' for human medicine (www.who.int/foodsafety/publications/antimicrobials-third/en/).

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Transparency declarations

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Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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Occurrence of high-level azithromycin-resistant *Neisseria gonorrhoeae* isolates in China

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Sir,

The most important threat to the future management and treatment of *Neisseria gonorrhoeae* infections is multidrug resistance. Resistance to ceftriaxone, the latest available first-line empirical monotherapy, is rising globally and confirmed treatment failures have been reported in several countries.[1] Therefore, dual antimicrobial therapies containing ceftriaxone and azithromycin are now the standard recommendation in Europe[2] and the USA.[3] This dual antimicrobial therapy is also currently being considered as the standard first-line therapy in China.[4] However, several countries have reported the occurrence of isolates with high-level azithromycin resistance,[5–7] which is defined by an MIC ≥256 mg/L. The emergence of these high-level azithromycin-resistant isolates poses a serious threat to the future efficacy of ceftriaxone and azithromycin dual antimicrobial therapy. As far as we are aware, this is the first report on the incidence and characterization of two high-level azithromycin-resistant isolates in China.

Both *N. gonorrhoeae* isolates were collected from patients at the Zhejiang Xiaoshan Hospital in Hangzhou, Zhejiang Province. Antimicrobial susceptibility testing of the *N. gonorrhoeae* isolates was performed using the agar dilution method. Both isolates showed high-level resistance to azithromycin, with MICs of 2048 mg/L. In addition, they were resistant to penicillin (MIC = 256 mg/L), ciprofloxacin (MIC = 16 mg/L) and tetracycline (MIC = 32 mg/L), but susceptible to ceftriaxone (MIC = 0.016–0.03 mg/L), cefixime (MIC = 0.03 mg/L) and spectinomycin (MIC = 16 mg/L). The genomes of the isolates were subsequently analysed by PCR amplification and sequencing to determine which azithromycin resistance determinants were present. Both isolates contained an A2059G mutation in loop V of all four 23S rRNA alleles encoded in the *N. gonorrhoeae* genome. This mutation has previously been associated with high-level azithromycin resistance of *N. gonorrhoeae* when present in minimally three of the four 23S rRNA alleles.[5] In addition, sequence analysis of the *mtrR* gene showed that both isolates contained a single base-pair deletion (A) in the 13 bp inverted repeat in the *mtrR* promoter and a G45D substitution