Comparison of the *in vitro* susceptibility of veterinary antibiotics with human antibiotics within aminoglycosides, β-lactam and fluoroquinolone antibiotic classes to highly resistant Gram-negative pathogens from human medicine

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The most common complication of cystic fibrosis (CF) in humans is the recurrence of chronic respiratory bacterial infections.[1,2] These infections are a major cause of morbidity and mortality[3] and are usually dominated by Gram-negative organisms, especially *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex (BCC) organisms. *P. aeruginosa* is the single most frequent pathogen in the adult patient population, leading to increased morbidity and mortality. Furthermore, infection with *B. cenocepacia* can be aggressive and eventually lead to fulminating cepacia syndrome, which is difficult to treat due to antibiotic resistance problems.

We have recently compared antibiotic resistance in patients with CF chronically infected with *P. aeruginosa* with *P. aeruginosa* from a non-CF source (invasive blood culture isolates).[4] Multidrug resistance (i.e., antibiotic non-susceptibility in two classes of three from aminoglycosides, β-lactams and fluoroquinolones) was observed in 15.0% of the CF patient population examined, while pan-resistance (i.e., antibiotic non-susceptibility in all three classes, aminoglycosides, β-lactams and fluoroquinolones) was observed in 9.5%, with all but one isolate being non-mucoid. Several other centres have reported similar antibiotic resistance problems, particularly in *P. aeruginosa* and BCC organisms, especially *B. cenocepacia*.[5–7]

The tangible consequence of such *in vitro* resistance, complicated by variable drug intolerences, is a profound difficulty in treating such patients with antibiotics. Therefore, exploration of novel antibiotic agents with susceptibility against these pan-resistant microflora is to be encouraged in order to allow drug development. For example, discovery of efficacious anti-*B. cenocepacia* antibiotics could reduce the stringency of contraindicating patients with *B. cenopacia* for lung transplantation and allow for the possible inclusion of CF patients with BCC in pharmaceutical drug trials.

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Presently, there are approximately 112 different antibiotic agents licensed in the UK for human and animal health, of which 35 (31%) are used exclusively in veterinary medicine. There are many shared classes of antibiotics between human medicine and veterinary medicine, but with different licensed antibiotic agents within each shared class. To date, we are not aware of any reports comparing the *in vitro* efficacy of antibiotic agents within individual classes of antibiotics, between human and veterinary medicine. Therefore, a study was performed examining differences in susceptibility in veterinary and human antibiotics within three classes of antibiotics, commonly employed in the treatment of CF patients, namely the aminoglycosides, the β-lactams and the fluoroquinolones.

Archived clinical isolates from adult patients with CF (n=50), consisting of *P. aeruginosa* (n=30) and *B. cenocepacia* (n=20) were obtained from the Northern Ireland Health and Social Care Microbiology Repository (MicroARK; www.microark.com). In addition, a further 13 Gram-negative isolates, consisting of members of the Enterobacteriaceae (*Escherichia coli*, n=8; *Enterobacter cloacae*, n=2; *Klebsiella oxytoca*, n=1; and *K. pneumoniae*, n=2) were examined, as comparators to the CF Gram-negative flora. All these Enterobacteriaceae had been previously isolated from patient blood cultures within the intensive care unit (ICU), Belfast City Hospital, Belfast Health and Social Care Trust. All isolates were recovered on Columbia blood agar (Oxoid, Basingstoke, UK), supplemented with 5% (v/v) defibrinated horse blood for 24 h at 37°C under aerobic conditions and passaged a further three times, prior to use.

Antibiotic susceptibility tests against 15 antibiotics were performed, consisting of eight sole veterinary antibiotic agents, as well as seven comparator human antibiotic agents, as detailed on Table 1. All antibiotic susceptibility discs were obtained from Oxoid, UK. Antibiotic susceptibility studies were performed on Mueller-Hinton agar supplemented with 5% (v/v) sheep blood (Oxoid PB0431). Following incubation at 37°C for 24 h, resulting zones of inhibition were measured and susceptibility was recorded as the resulting zone size (mm).

Mean zone sizes (mm) of the 63 isolates against the 15 antibiotic agents are shown in Table 1. Overall, the CF isolates were markedly more resistant than the ICU Enterobacteriaceae, possibly reflecting the chronic exposure of the CF pathogens to the classes of antibiotics tested. With the CF pathogens and within the aminoglycosides, tobramycin had the greatest activity, compared with any of the three aminoglycoside veterinary antibiotics (apramycin, framycetin and kanamycin). There was no activity with any of the CF pseudomonads and kanamycin. Tobramycin was the least effective with members of the Enterobacteriaceae, whereas the veterinary antibiotics showed superior activity in the order framycetin>kanamycin>apramycin.

With the β-lactam agents, there was markedly better antipseudomonal activity with the third-generation veterinary cephalosporins, cefoperazone and ceftiofur than with the human third-generation agents cefotaxime and cefuroxime. There was no activity with cloxacillin with any Gram-negative isolate tested. Similarly, there was excellent activity with the veterinary cephalosporins against members of the Enterobacteriaceae, than with the human cephalosporins, with larger zone sizes being observed with the veterinary cephalosporins.

Table 1. Description of organisms and veterinary and human antibiotics examined.

			Mean zone of inhibition (mm)		
Class of antibiotic	**Veterinary or human use**	**Antibiotic (μg)**	***Pseudomonas aeruginosa* (*n*=30)**	***Burkholderia cenocepacia* (*n*=20)**	**Enterobacteriaceae (*n*=13)**
Aminoglycosides	Veterinary	Apramycin (15)	9.7	0	18.3
		Framycetin (100)	17.2	0	22.8
		Kanamycin (30)	0	0	21.5
	Human	Tobramycin (10)	17.8	0	17.9
β-lactams	Veterinary	Cloxacillin (5)	0	0	0
		Cefoperazone (30)	19	0	28.1
		Cefovecin (30)	0	3.8	26.0
		Ceftiofur (30)	11	3.6	28.8
	Human	Ampicillin (10)	0	0	6.5
		Aztreonam (30)	25	5	32.5
		Cefotaxime (30)	3.8	0	24.7
		Cefuroxime (30)	0	3.8	26.0
Fluoroquinolones	Veterinary	Enrofloxacin (5)	12.5	2.5	22.7
	Human	Ciprofloxacin (5)	17.8	4.5	25.4
		Moxifloxacin (1)	4.7	0	18.5

0: no zone of inhibition (i.e., growth of bacteria to edge of antibiotic disc).

Within the fluoroquinolines, the veterinary agent enrofloxacin had less activity against *P. aeruginosa* than ciprofloxacin ($P=0.001$), but had better antipseudomonal activity than moxifloxacin ($P<0.0001$). Equally, ciprofloxacin had much superior activity against *P. aeruginosa* than moxifloxacin ($P<0.0001$).

This study was undertaken to compare the potential activity of veterinary antibiotics against Gram-negative pathogens with activity of human antibiotics, within the same class of antibiotic. To date, we are not aware of any report comparing potential activity of exclusively veterinary antibiotics within the same class of antibiotics from human medicine.

The emerging problem of global antibiotic resistance is now forcing science to explore alternative routes to *de novo* antibiotic drug discovery. Such antibiotic resistance can be clearly mapped at the local, national and international levels both within animal as well as human patient populations. While there is a misconception that the availability of antibiotics is relatively abundant, several hundred antibiotic formulations, both branded and non-branded, appear to be available. While this is true, the majority of these can be placed into relatively few (<10) classes of antibiotic agents, which may be shared between human and veterinary medicine. In an ideal clinical environment, we should attempt to segregate the use of antibiotics, with a set uniquely for veterinary use and a set solely for human medicine purposes. Sadly, this is not the case, even though it may be the intention or aspiration of both human as well as veterinary medicine. The relatively few classes of antibiotics and the emergence of antibiotic resistance no longer allows for the relative luxury of attempting to segregate antibiotics and more importantly the antibiotic classes to either human and veterinary medicine.

However, in an attempt to preserve antibiotic efficacy for the treatment of infections, certain antibiotics have been reserved exclusively for human or veterinary use. This is regulated by licensing legislation within the pharmaceutical industries. While these agents may be protected for targeted use in either humans or animals, generally they will at least share an antibiotic class. For instance, Table 1 shows that within the aminoglycosides, apramycin, framycetin and kanamycin are listed as veterinary drugs, while tobramycin is listed as a human antibiotic.

This framework of licensing antibiotic agents exclusively for human medicine or veterinary medicine therefore creates a scenario that certain antibiotic agents within veterinary medicine would never have been exposed to human pathogens, and therefore veterinary antibiotics may retain some additional coverage with resistant human pathogens.

Overall, veterinary antibiotics showed better activity with members of the Enterobacteraceae with the antibiotic classes aminoglycosides and the β-lactams, than their human comparators. This, however, was not the case with the fluoroquinolones, where the human form (i.e., ciprofloxacin) had better activity than the veterinary enrofloxacin. These data indicate that there will be a need for dialogue between veterinarians and human physicians, as well as with the medicine regulators, especially where veterinary pharmaceuticals offer an opportunity which cannot be fulfilled from within human medicine.

In order to understand the differences in antibiotic activity within the same class of antibiotics, Figure 1 compares the chemical structure of the veterinary antibiotic with its closest relative from the same class of antibiotics in human medicine.

As yet, we have not performed this study the other way around, namely comparing the efficacy of human versus veterinary antibiotics within the same class, against a collection of highly resistant veterinary bacterial pathogens. The current study therefore raises interesting moral and

ethical considerations. The avoidance of employing the same antibiotic in human medicine and simultaneously in veterinary medicine, through careful regulation and licensing mechanisms, has created an excess of susceptibility potential for the exploitation of veterinary antibiotics in human medicine. One could therefore justifiably argue that this excess in antibiotic susceptibility should first be allowed to be exploited within veterinary medicine. Is it therefore morally acceptable to raid/plunder the veterinary antibiotics cupboard for an enhanced human outcome? This is an issue that society will now have to debate; however, such debates become possible only through the generation of data, such as those which are included in the study reported here.

In conclusion, these data indicate that veterinary antibiotic agents within the same class of antibiotics may perform better in terms of improved efficacy over their most closely related human antibiotics. This should be carefully monitored and explored further, especially when human medicine encounters major antibiotic resistance problems and is seeking alternative agents to combat highly resistant infection. □

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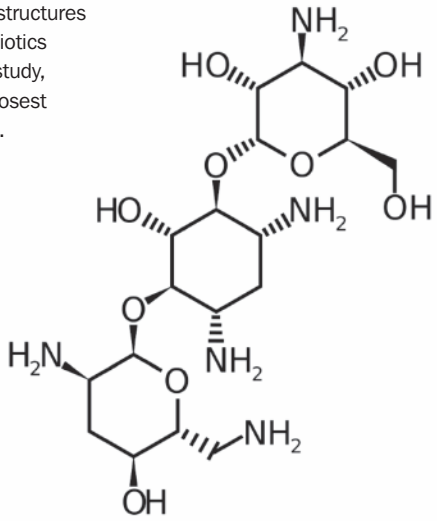
HUMAN AMINOGLYCOSIDE

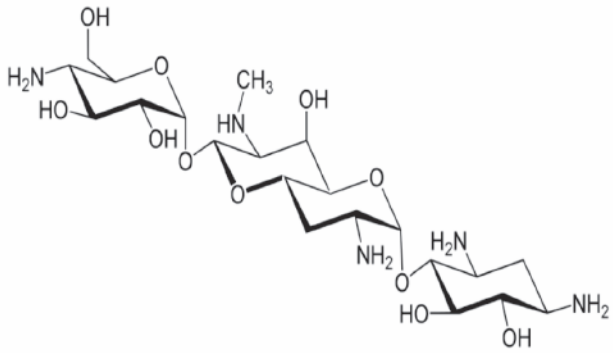
Fig. 1. Chemical structures of veterinary antibiotics employed in this study, along with their closest human equivalent.

Tobramycin

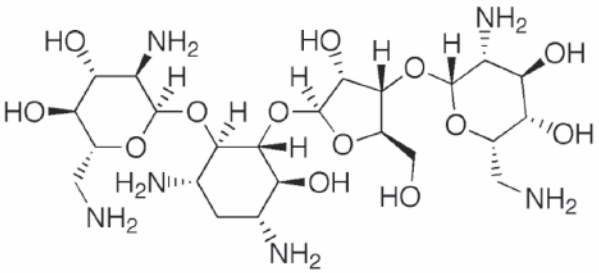


VETERINARY AMINOGLYCOSIDES

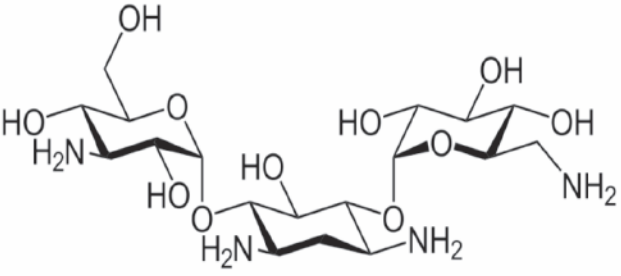
Apramycin



Framycetin

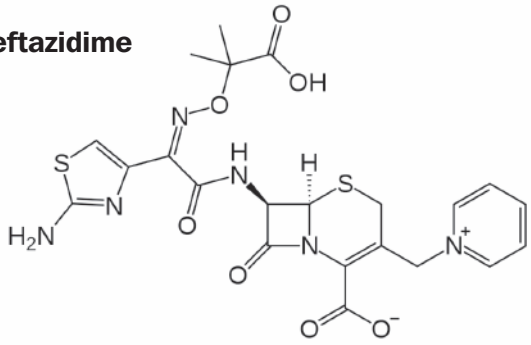


Kanamycin



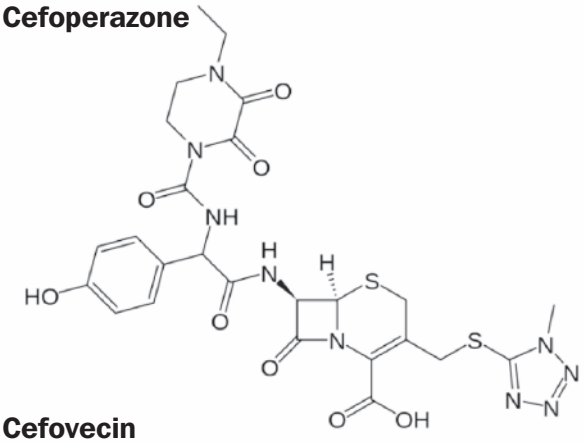
HUMAN THIRD-GENERATION CEPHALOSPORIN

Ceftazidime

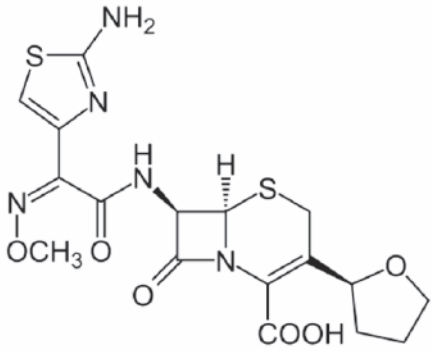


VETERINARY THIRD-GENERATION CEPHALOSPORINS

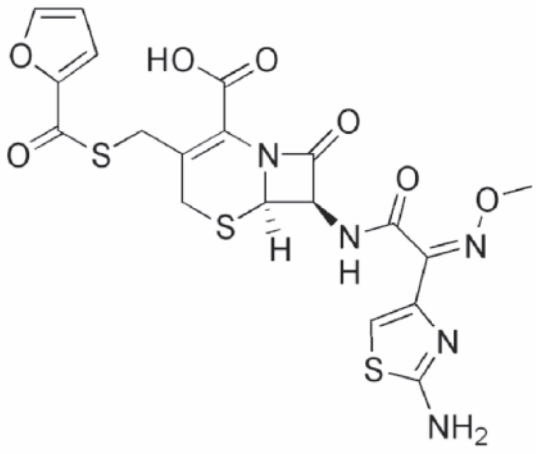
Cefoperazone



Cefovecin

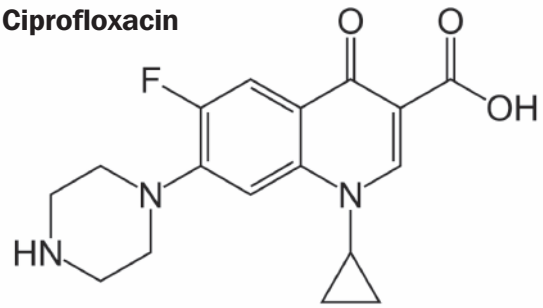


Ceftiofur



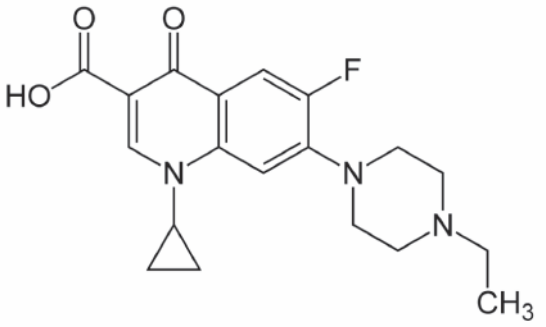
HUMAN FLUOROQUINOLONE

Ciprofloxacin



VETERINARY FLUOROQUINOLONE

Enrofloxacin



Tobramycin (2S,3R,4S,5S,6R)-4-amino-2-{[(1S,2S,3R,4S,6R)-4,6-diamino-3-{[(2R,3R,5S,6R)-3-amino-6-(aminomethyl)-5-hydroxyoxan-2-yl]oxy}-2-hydroxycyclohexyl]oxy}-6-(hydroxymethyl)oxane-3,5-diol)
Apramycin (2R,3R,4R,5S,6R)-5-amino-2-[((1R,2R,3R,4R,6R,8R)-8-amino-9-[(1R,2S,3R,4R,6R)-4,6-diamino-2,3-dihydroxy-cyclohexyl]oxy-2-hydroxy-3-methylamino-5,10-dioxabicyclo[4.4.0]dec-4-yl)oxy]-6-(hydroxymethyl)oxane-3,4-diol)
Framycetin (2R,3S,4R,5R,6R)-5-amino-2-(aminomethyl)-6-[(1R,2R,3S,4R,6S)-4,6-diamino-2-[(2S,3R,4S,5R)-4-[(2R,3R,4R,5S,6S)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-yl]oxy-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3,4-diol)
Kanamycin (2-(aminomethyl)-6-[4,6-diamino-3-[4-amino-3,5-dihydroxy-6-(hydroxymethyl)tetrahydropyran-2-yl]oxy-2-hydroxy-cyclohexoxy]-tetrahydropyran-3,4,5-triol)
Ceftazidime (6R,7R,Z)-7-(2-(2-aminothiazol-4-yl)-2-(2-carboxypropan-2-yloxyimino)acetamido)-8-oxo-3-(pyridinium-1-ylmethyl)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate)
Cefoperazone (6R,7R)-7-[(2R)-2-{[(4-ethyl-2,3-dioxopiperazin-1-yl) carbonyl]amino}-2-(4-hydroxyphenyl)acetamido]-3-{[(1-methyl-1H-1,2,3,4-tetrazol-5-yl)sulfanyl]methyl}-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene-2-carboxylic acid)
Cefovecin ((7R)-7-([(2Z)-2-(2-amino-1,3-thiazol-4-yl)-2-methoxyimino acetyl]amino)-8-oxo- 3-[(2S)-oxolan-2-yl]- 5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid)
Ceftiofur (6R,7R)-7-[[(2Z)-2-(2-Amino-1,3-thiazol-4-yl)-2-methoxy iminoacetyl]amino]-3-(furan-2- carbonylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid)
Ciprofloxacin (1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid)
Enrofloxacin (1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid)7