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Antimicrobial resistance in *Brachyspira* – An increasing problem for disease control

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ABSTRACT

Across all bacterial species the continuing reduction in susceptibility to antimicrobial agents is a critical and increasing threat for disease control. This mini-review outlines the extent of this problem amongst anaerobic intestinal spirochaetes of the genus *Brachyspira*, of which there are currently nine officially recognised species. These include some important pathogens that may cause colitis with diarrhoea and/or dysentery in various mammalian and avian species, but most notably in pigs and in adult chickens. The most economically significant pathogen is *Brachyspira hyodysenteriae,* the spirochaete which causes swine dysentery in countries throughout the world. Control of infections with *Brachyspira* species has long relied on the prophylactic or therapeutic use of antimicrobials, but increasingly strains with reduced susceptibility and sometimes multidrug resistance to previously effective antimicrobial agents are being encountered. In this mini-review we outline these problems and explain the extent and molecular basis of the emerging resistance. Future control will rely on developing and applying standardised methods for measuring antimicrobial susceptibility; improving surveillance of resistance using traditional phenotypic as well as genomic analysis of known resistance determinants; improving understanding of the molecular basis of resistance to different drug classes; improving farmer and veterinarian education about prudent antimicrobial use so as to reduce selective pressure on the emergence of resistance; and developing alternatives to antimicrobials as a means to control these infections.

1. The genus *Brachyspira*

The genus *Brachyspira* comprises a diverse group of oxygen-tolerant anaerobic spirochaetes that inhabit the large intestines of a variety of mammalian and avian hosts. The genus currently includes nine species on the list of officially approved names (Table 1), as well as several unofficially suggested species, most of which appear to be commensals. Many but not all of the official species are confirmed pathogens of animals based on fulfilment of Koch's postulates, although the species and individual strains vary widely in their pathogenic potential in susceptible hosts. An update on the *Brachyspira* species and associated diseases recently has been presented by Looft and Stanton (2018). Briefly, the most common and important pathogenic species include *Brachyspira hyodysenteriae*, the originally-described agent of swine dysentery (SD); *Brachyspira pilosicoli*, the agent of intestinal spirochaetosis in pigs, poultry, dogs, horses and other species - including human beings; *Brachyspira intermedia* and *Brachyspira alvinipulli*, both of which are agents of avian intestinal spirochaetosis; and the newly recognized species *Brachyspira suanatina* and *Brachyspira hampsonii* that can cause similar clinical signs and lesions to *B. hyodysenteriae* in pigs. This article places most emphasis on *B. hyodysenteriae* and *B. pilosicoli*, since the bulk of available data about antimicrobial susceptibility relates to these two species, and they can cause significant clinical problems and economic loss in pigs and/or adult poultry.

2. Prevention and control of *Brachyspira* infections

The main source of new infections with *Brachyspira* species comes

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Table 1
Disease associations of the nine officially recognised *Brachyspira* species.

Species	Main host species	Associated diseases
B. hyodysenteriae	Pigs, feral waterbirds?	Swine dysentery
B. hampsonii	Pigs, feral waterbirds	Swine dysentery
B. suanatina	Feral ducks, pigs	Swine dysentery
B. pilosicoli	Many species; including pigs, chickens, dogs, horses and humans	Intestinal spirochaetosis (porcine/avian/human)
B. intermedia	Chickens, pigs	Avian intestinal spirochaetosis; mild colitis in pigs?
B. innocens	Pigs, chickens, rats	Not recorded
B. murdochii	Pigs, chickens, rats	Mild colitis in pigs?
B. alvinipulli	Chickens	Avian intestinal spirochaetosis
B. aalborgi	Humans and other primates	Human intestinal spirochaetosis

from other colonised animals, and consequently all new stock introduced into farms should be screened for carriage of these and other pathogens, and subjected to quarantine. A number of the *Brachyspira* species that are listed in Table 1 have more than one potential host species, and cross-species transmission may occur. Consequently, strict biosecurity measures are required on farms to reduce such transmission by preventing contact between the farmed animal species and potential reservoirs of infection such as wild birds and rodents. Although the *Brachyspira* species have their main ecological niches in the large intestine, they can survive outside the host for days or weeks under moist anaerobic conditions (such as in slurry pits or ponds). Control strategies within farms include limiting possible exposure to these materials, and ensuring good management and hygiene to reduce transmission between groups of animals, particularly of different ages. The physicochemical and microbial environment in the large intestine can influence the growth of *Brachyspira* species, but currently there are no universal recommendations about how to modulate this in a way that reduces colonisation either through modifications to the diet (Pluske and Hampson, 2009) or by other means such as feeding animals with probiotic bacteria (Mappley et al., 2013). Despite much research having been undertaken, no suitable protective vaccines against *B. hyodysenteriae* or other pathogenic *Brachyspira* species are available commercially. Antimicrobial agents, applied both therapeutically and prophylactically, still remain the major means for treatment and control of SD and intestinal spirochaetosis in pigs and other species. Huge quantities of antimicrobials that are largely administered orally are used for this purpose, and this produces pressure for selection of resistant *Brachyspira* isolates and other off-target enteric bacterial species. The steady trend for *Brachyspira* isolates to show a reduction in susceptibility to antimicrobials is a major global problem, particularly where resistant pathogenic spirochaetes are established in large integrated pig production systems, including in otherwise high health status breeding herds that supply animals to other herds. There are only a few antibiotics that are still licenced for use to control *Brachyspira* infections in pigs, and control of infections in laying chickens has been limited by antimicrobial withholding periods imposed for table eggs, and a lack of suitable registered products with short withholding periods.

3. Antimicrobials used for *Brachyspira* control

Brachyspira species are intrinsically resistant to a number of antimicrobials: these include spectinomycin, colistin, vancomycin, rifampicin, spiramycin and flavomycin, all of which have been incorporated in varying concentrations into blood agar or broth media to inhibit the intestinal microbiota and make them selective for isolation of the slow-growing *Brachyspira* from faeces, intestinal contents or intestinal wall scrapings (Jenkinson and Wingar, 1981; Kunkle and Kinyon, 1988; Lugsomya et al., 2012).

The antimicrobials to which pathogenic *Brachyspira* species isolated from animals have been reported to be susceptible include dimetridazole and metronidazole (imidazoles), lincomycin (lincosamides), tylosin and tylvalosin (macrolides), tiamulin and valnemulin (pleuromutilins), carbadox and olaquindox (quinoxalines), virginiamycin (streptogramins), and salinomycin and monensin (ionophores). Human intestinal spirochaetosis caused by *B. pilosicoli* and/or *B. aalborgi* is most frequently treated with penicillins or metronidazole. In animals the majority of antimicrobial agents are administered in-feed as preventatives during susceptible phases of the production animal cycle, particularly in pig herds with endemic SD. Some of these antimicrobials can result in toxicity if used in combination [e.g. tiamulin with salinomycin, or with other ionophores] (Wendt et al., 1997). Additionally, some of these agents may be effective against infections with *Brachyspira* spp. also by virtue of their effects on other components of the colonic microbiota that normally act synergistically towards *Brachyspira* species isolates (Costa et al., 2014; Looft et al., 2014). This phenomenon may explain the continued effectiveness of lincomycin combined with spectinomycin as a control agent, despite widespread resistance to lincomycin among field strains of *B. hyodysenteriae*. The same applies to tylosin, where demonstrated *in vitro* resistance among *B. hyodysenteriae* isolates is extremely common. Additionally, some of the agents that are effective for control of *Brachyspira* infections (dimetridazole, carbadox, olaquindox) are no longer registered for use in most countries due to concerns regarding their carcinogenic potential, or they have been removed from animal feed due to their classification as antimicrobial growth promoters. Furthermore, low levels of carbadox and metronidazole have been shown to be potent inducers of VSH-1 prophage-like elements, which promote lateral gene transfer among *Brachyspira* and may contribute to the highly recombinant nature of the *Brachyspira* genome (Stanton et al., 2008). There is some suggestion that the widescale removal of antimicrobials with label claims of growth promotion in animal feeds has resulted in an increased prevalence of *Brachyspira* infections, although studies are limited and the results have been somewhat equivocal (Laine et al., 2004).

4. *Brachyspira* antimicrobial susceptibility testing

Currently there are no Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardised methods for antimicrobial susceptibility testing of *Brachyspira* species. There is a CLSI guideline for testing of infrequently isolated or fastidious bacteria isolated from animals (CLSI, 2017), although the scientific basis for the *Brachyspira* guidelines are debatable. Susceptibility testing using either agar dilution or broth dilution is accepted, but disc diffusion is not recommended for anaerobic *Brachyspira* species (CLSI, 2018). Antimicrobial gradient strip tests using different antimicrobials have been investigated for four *Brachyspira* species, but the results were more ambiguous than those obtained with broth dilution for determining minimum inhibitory concentrations (MIC) (Mirajkar and Gebhart, 2016). A number of non-standardised anaerobic blood agar dilution methods have been described (Karlsson et al., 2003), but test conditions have varied considerably and the results are not easily comparable. Some notable differences between methods that may influence the results include the antimicrobials tested, their chemical formulations; the dilutions used; the type, composition and volume (for broth dilution) of the media; the species of origin of the blood or serum used; the incubation time; the incubation temperature; the gaseous environment; the inoculum size; the spirochaete growth phase; and end point determination (e.g. rather than recording an absence of growth on the plate, using a lack of haemolysis, which may only reflect inhibition of haemolysin production) (Kulathunga and Rubin, 2017). MICs generally are lower by around one dilution when using the broth dilution method compared to agar dilution (Rohde et al., 2004).

Broth dilution assays using VetMIC Brachy panels that are commercially available from the National Veterinary Institute, Uppsala, Sweden are routinely used by the Swedish Veterinary Antimicrobial Resistance Monitoring program (SVARM), and have been used in a number of scientific studies in other countries (Pringle et al., 2012). The panels mainly have been used for testing the agents of SD. In this system, antimicrobial agents are dried in wells in tissue culture trays and a 0.5 ml volume of brain heart infusion broth with 10% foetal calf serum is added to each well with the test strain and incubated anaerobically with agitation. This technique is very robust, with clear end points and little variation in MIC resulting from changes in inoculum density and incubation time (Karlsson et al., 2002). In some laboratories broth microdilutions using microdilution trays with a maximum volume per well of 0.1 ml have been used for MIC testing (Herbst et al., 2014; Mahu et al., 2017). The *B. hyodysenteriae* type strain B78$^{\mathrm{T}}$ has been evaluated and recommended for use as the control strain for *Brachyspira* MIC testing (Pringle et al., 2006a); however, this strain has low MICs for tiamulin, and this limits its use as a quality control (Rohde et al., 2004).

4.1. *MICs and cut-off values*

When examining the distribution of MIC values for a large set of bacterial isolates against a given antimicrobial it may be possible to determine a breakpoint or epidemiological cut off value (ECOFF) that differentiates the wild type population from non-wild type isolates. Wild type strains do not harbor acquired or selected resistance, whilst the non-wild type isolates have higher MICs than the wild type and show evidence of 'microbiological resistance' or reduced susceptibility. The ECOFF value is distinct from the 'clinical breakpoint' which is usually higher and is the threshold above which clinical resistance occurs. Strains with MIC values above the clinical breakpoint are unlikely to be successfully controlled with the drug tested.

Clinical breakpoints for *B. hyodysenteriae* and *B. pilosicoli* that have been suggested by different authors for different antimicrobials are presented in Table 2, together with ECOFFs for *B. hyodysenteriae* using the commercial VetMIC Brachy panels (Pringle et al., 2012). The values varied between the studies, as did the methods used to establish the MICs. One generally accepted finding is that the clinical breakpoint of > 4 µg/ml set for tiamulin by Rønne and Szancer (1990) was too high, and a value of > 2 µg/ml using the VetMIC Brachy panel is more realistic (SVARM, 2014). Although Pringle et al (2012) also tested 324 *B. pilosicoli* isolates they did not propose separate ECOFF values for this species. In view of the absence of published data for this and other pathogenic *Brachyspira* species, the ECOFF values for *B. hyodysenteriae* also may be cautiously used for the other species.

5. Reduced susceptibility to antimicrobials amongst *Brachyspira* isolates

Since the discovery of *Brachyspira* species in the early 1970s there have been numerous reports on progressive temporal increases in MICs of isolates to various antimicrobial agents. To circumscribe this broad topic, we present a summary of MIC data on the antimicrobial agents that have most commonly been used to control *Brachyspira* species in animals: these include carbadox, lincomycin, tylosin, tyvalosin, tiamulin and valnemulin. Carbadox has now been removed from use in most countries because of genotoxicity concerns, but is still used in the USA and hence available data are presented. Summaries of published data (MIC_{50}, MIC_{90} and MIC range) for these six antimicrobials for isolates of *B. hyodysenteriae*, *B. pilosicoli* and other *Brachyspira* species published in the 15-year period 2003–2018 are outlined in Tables 3–5, respectively. The tables also indicate the percentage of isolates that were non-wild type in relation to the different agents in each study. Some of these studies also examined other antimicrobial agents, but for brevity the results for these are not presented.

5.1. *Early history of development of reduced susceptibilities (pre-2004)*

Brachyspira hyodysenteriae was identified as the agent of SD at the beginning of the 1970s, and a lack of susceptibility to tylosin was first noted during the SD eradication programme undertaken in Germany in the late 1970s (Blaha et al., 1987). Subsequently isolates with high MIC values to tylosin, other macrolides such as tylvalosin, and to lincomycin have become widespread in many countries (see below). High MIC values to tylosin and lincomycin also has been noted among avian intestinal spirochaetes (*B. intermedia* and *B. pilosicoli*: Tables 4 and 5).

Valnemulin and its progenitor tiamulin are pleuromutilins that show excellent activity against *B. hyodysenteriae* and other *Brachyspira* spp. at relatively low concentrations. Increases in MICs to tiamulin were noted in field isolates of *B. hyodysenteriae* in Australia and in Hungary in the early to mid-1990s (Buller and Hampson, 1994; Molnár, 1996). An investigation of reduced susceptibility to tiamulin among British and German isolates of *B. hyodystenteriae* revealed that the isolates were not clonal but rather represented the independent evolution of increased resistance in isolates on distinct farms in each country (Karlsson et al., 2004a). Subsequently other countries reported an increase in tiamulin and valnemulin MICs in *B. hyodysenteriae*, particularly in the Czech Republic (Lobová et al., 2004).

5.2. *Inter-country spread of B. hyodysenteriae clones with increased MICs to tiamulin*

In recent years, evidence been reported from Europe on inter-country spread of clonal lineages of *B. hyodysenteriae* with increased

Table 2

Some proposed breakpoints for interpretation of MIC values (µg/ml) with six commonly used antimicrobials for *B. hyodysenteriae* and *B. pilosicoli.*

	Method for AST[a]	Breakpoint	Carbadox	Lincomycin	Tylvalosin	Tylosin	Tiamulin	Valnemulin	Publication
B. hyodysenteriae	AD	Clinical	–	> 36	–	> 4	> 4	–	Rønne and Szancer, 1990
	BD	Clinical	–	> 50-100	> 16-32	> 16-32	> 0.5-1	> 0.125-0.25	Burch, 2005
	AD	Clinical		–	–	–	≥1	–	Vyt and Hommez, 2006
	BD*	ECOFF	–	> 1	> 1	> 16	> 0.25	> 0.125	Pringle et al., 2012
	BD*	Clinical	–	–	–	> 16	> 2	–	SVARM, 2014
B. pilosicoli	AD	Clinical	≥1	≥75	–	–	≥2	–	Duhamel et al., 1998
	BD*	Clinical	–	–	–	> 16	> 2	–	SVARM, 2014

[b] ECOFF, epidemiological (wild type) cutoff; 'Clinical', indicates resistance breakpoint.

[a] AST: antimicrobial susceptibility testing. ADS, agar dilution. BD, broth dilution. Testing with commercial VetMIC Brachy marked with an asterisk*.

Table 3

Summary of country-specific MIC results (μg/ml) for *B. hyodysenteriae* isolates from pigs for the six antimicrobial agents most commonly used to control *Brachyspira* infections (publications from 2003 to 2018).

Country	Bacterial isolating year	Number of isolates	method for AST[a]	carbadox		lincomycin		tyvalosin		tylosin		tiamulin		valnemulin		Reference
				MIC_{50} MIC_{90} Range	% non-wild type[c]	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	
Sweden	1990-1999	72	BD	0.031 0.063 < = 0.004-0.125	0	–	–	–	–	> 256 > 256 < = 2-256	58.3	−0.063, 0.25, < = 0.016-0.5	1.4	–	–	Karlsson et al., 2003
Japan	2002	37	AD	–	–	– 50 25-100	100	–	–	– > 100, > 100	100	– 0.4, < 0.1-1.6	–	–	–	Uezato et al., 2014
Czech Republic	1997-2001	100	AD	–	–	–	–	–	–	–	–	0.25 2 < = 0.031-16	39	0.125 4 < = 0.031-16	48	Lobová et al., 2004
Germany	1989-2002	323	BD	–	–	– – –	–	– –	–	– –	–	0.5 4 0.031-8	59	0.125 2 0.031-4	48.6	Rohde et al., 2004
Spain	2000-2007	108	BD	–	–	16 128 2- > 128	100	–	–	> 256 > 256 < = 4- > 256	99.1	0.25 > 2 < = 0.016- > 2	43.5	0.125 2 < = 0.016-> 2	48	Hidalgo et al., 2009
Japan	1985-2000	72	AD	–	–	32 > 128 0.25-128	91.7	–	–	> 128 > 128 4- > 128	87.5	0.125 2 < = 0.06-8	20.8	< = 0.06 8 < = 0.06-8	22.2	Ohya and Sueyoshi, 2010
USA	2008-2010	24	AD	– 0.3 0.008-0.06	0	−6 4 1-64	–	–	–	– – –	–	– 0.5 0.125-4	–	– 0.5 0.125-2	–	Clothier et al., 2011
Czech Republic	2000-2005	35	AD	–	–	64 128 4- > 128	100	–	–	> 128 > 128 1- > 128	91.4	32 > 6 16- > 64	100	32, > 64 8- > 64	100	Sperling et al., 2011
Spain	2008-2009	87	BD*	–	–	16 > 6 1- > 64	98.9	4 16 2-32	88.5	> 128 > 128 16- > 128	98.9	1 8 < = 0.063-8	74.7	1 4 < = 0.03-.4	74.7	Hidalgo et al., 2011
South Korea	2003-2005	5	AD	–	–	– – –	–	–	–	128 > 256 128- > 26	100	0.125 8 < 0.25-8	25	–	–	Lim et al., 2012
Sweden	1990-2010	532	BD*	–	–	32 32 < = 0.5-> 64	73.6	4 16 < = 0.25-> 32	72.9	> 128 > 128 < = 2- > 128	74.1	0.125 0.25 < = 0.016-2	8.1	< = 0.031 0.063 < = 0.031-1	4.8	Pringle et al., 2012
Poland	2006-2010	21	BD*	–	–	16 16 2-32	95.2	–	–	> 128 > 128 16- > 128	95.2	0.5 1 0.25-1	85.7	1 2 0.063	71.4	Zmudzki et al., 2012
Czech Republic	1997-2006	202	AD	–	–	64 128 2-128	68.6-82.2	25 100 3.125-200	0-64.3	> 128, > 128 1- > 128	98	0.5 16 < = 0.03-16	0-42.8	0.25 16 < = 0.03-> 16	0-60.7	Prášek et al., 2014
Germany	2003-2012	103	BD	–	–	16 32 < = 0.063-> 64	92.2	–	–	> 128 > 128 < = 0.125- > 128	93.2	0.5 8 < = 0.063- > 64	53.4	0.125 > 4 < = 0.004-> 4	45.6	Herbst et al., 2014

(*continued on next page*)

Table 3 (*continued*)

Country	Bacterial isolating year	Number of isolates	method for AST[a]	carbadox		lincomycin		tyvalosin		tylosin		tiamulin		valnemulin		Reference
				MIC_{50} MIC_{90} Range	% non-wild type[c]	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	
Italy	2003-2012	103	BD	–	–	–	–	–	–	–	–	8 6 < 0.063- > 8	73.8	4 8 < 0.031- > 4	76.7	Rugna et al., 2015
Japan	2009	29	AD	< 0.013 < 0.013 < 0.013-0.05	0	3.13 12.5 0.78-25	58.6	25 50 0.39-100	82.8	> 100 > 100 < 0.1- > 100	82.8	1.56 6.25 0.1-12.5	96.6	3.13 6.25 0.39-6.25	93.10	Kajiwara et al., 2016
USA	2009-2014	40	BD* AD[b]	0.008 0.25 0.002-0.25	0	16 32 < = 0.5- > 64	75	–	–	> 128 > 128 < = 2- > 128	75	< = 0.063 2 < = 0.063-8	25	< = 0.031 1 < = 0.031-2	27.5	Mirajkar et al., 2016
Switzerland	2009-2015	30	BD*	–	–	16 32 < = 0.5-32	70	4 8 0.25-4	66.7	> 128 > 128 < = 2- > 128	63.3	< = 0.063 0.125 < = 0.063-0.5	0	– – –	–	Kirchgässner et al., 2016
Australia	2014-2016	46	AD	–	–	= > 36- > 72 = > 72 < 2-= > 72	60.1	–	–	= > 100 = > 100 = > 1 > 5- = > 100	69.6	= > 0.25 < 0.5 = > 4 > 8 < 0.25-= > 8	73.9	–	–	La et al., 2016
Belgium	2010-2015	30	BD	–	–	16 32 0.25-64	86.7	–	–	> 128 > 128 2- > 128	80	0.5 4 < = 0.004-8	53.3	0.25 2 < = 0.002-8	56.7	Mahu et al., 2017
Brazil	2012-2013	22	BD*	–	–	64 > 64 2- > 64	100	16 32 1-32	95.5	> 128 > 128 4- > 128	95.5	8 8 < = 0.063- > 8	90.9	2 > 4 < = 0.031- > 4	90.9	Daniel et al., 2017
Germany	1990-2016	116	BD	–	–	–	–	–	–	–	–	2 – < = 0.06- > 16	39.1	0.5 – < = 0.004- > 4	39.1	Joerling et al., 2018
Taiwan	Recent	37	AD	< = 0.125 0.25 –	–	32 > 256 < = 0.125- > 256	97.3	–	–	> 256 > 256 8- > 256	86.5	1 32 < = 0.125-64	> 51	–	–	Yeh et al., 2018
Switzerland	2010-2017	51	BD*	–	–	16 32 0.25-64	82.3	4 8 0.125-64	88.2	> 128 > 128 1- > 128	84.3	< 0.031 < 0.031 < 0.031-0.25	0	–	–	García-Martín et al., 2018

[a] AST: Antimicrobial susceptibility testing. BD, broth dilution; AD, agar dilution. Studies using the commercial VetMIC Brachy panel are marked with an asterisk*.

[b] BD method, except AD for carbadox.

[c] The percentage recorded as non-wild type for each antimicrobial was determined using the epidemiological (wild-type) cut-off values described by Pringle et al. (2012).

Table 4

Summary of country-specific MIC results (μg/ml) for *B. pilosicoli* isolates for the six antimicrobial agents most commonly used to control *Brachyspira* infections (publications from 2003 to 2018).

host	Country	Bacterial isolating year	Number of isolates	method for AST[a]	carbadox		lincomycin		tyvalosin		tylosin		tiamulin		valnemulin		Reference
					MIC_{50} MIC_{90} Range	% non-wild type[b]	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	MIC_{50} MIC_{90} Range	% non-wild type	
pig	Sweden	1990-2001	41	BD	0.063 0.125 0.008-0.5	–	–	–	–	–	> 256 > 256 4- > 256	61.9	0.125 2 0.031-32	56.1	–	–	Karlsson et al., 2004b
pig	Sweden	2002-2003	93	BD	–	–	–	–	–	–	32 > 128 < = 2- > 128	38.3	0.125 8 0.031- > 64	27.9	–	–	Pringle et al., 2006b
chicken	Australia, USA, The Netherland	1996-1999	17	AD	–	–	10 50 < 1-50	58.8	–	–	20 > 100 < 4- > 100	47.1	< 0.1 1 < 0.1-1	17.6	–	–	Hampson et al., 2006
pig	USA	2008-2010	6	AD	– 0.03 0.008-0.03	0	– 64 1-64		–	–	– – –	–	– 0.5 0.25-1	–	– 0.5 0.125-1	–	Clothier et al., 2011
pig	Sweden	2002-2010	324	BD*	–	–	8 32 < = 0.5-64	67.5	2 > 32 0.5-32	60.3	128 > 128 < = 2- > 128	59.3	0.125 16 < = 0.063- > 8	30.2	0.063 1 < = 0.031- > 4	29.4	Pringle et al., 2012
pig	USA	2009-2014	20	BD* AD	0.008 0.016 0.004- 0.016	0	32 64 < = 0.5- > 64	91.7	–	–	> 128 > 128 8- > 128	87.5	0.5 > 8 < = 0.063- > 8	54.2	0.25 2 < = 0.031-4	58.3	Mirajkar et al., 2016
pig	Taiwan	Recent	5	AD	< = 0.125 < = 0.125 < = 0.125	0	32 32 16-64	100	–	–	> 256 > 256 > 256	100	< = 0.125 0.5 < = 0.12-15	40	–	–	Yeh et al., 2018

[a] BD, broth dilution; AD, agar dilution. Studies using the commercial VetMIC Brachy panel are marked with an asterisk*.

[b] The percentage recorded as non-wild type to each antimicrobial was determined using the epidemiological (wild-type) cut-off values described by Pringle et al. (2012).

Table 5
Summary of country-specific MIC results (μg/ml) for *Brachyspira* spp. other than *B. hyodysenteriae* and *B. pilosicoli* for the antimicrobial agents most commonly used to control *Brachyspira* infections (publications from 2003 to 2018).

Bacterial strains	host	Country	Bacterial isolating year	Number of isolates	method for AST[a]	carbadox		lincomycin		tyvalosin		tylosin		tiamulin		valnemulin		Reference
						MIC$_{50}$ MIC$_{90}$ Range	% non-wild type[b]	MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	
B. intermedia	pig	Sweden	1990-1999	20	BD	0.008 0.03 0.008-0.06	0	–	–	–	–	4 > 256 < = 2-26	30.0	0.06 0.25 < = 0.016-2	5	–	–	Karlsson et al., 2003
B. intermedia	chicken	Australia, USA, The Netherlands	1996-1999	25	AD	–	–	< 1 5 < 1-50	48	–	–	< 4 > 100 < 4- > 100	28	1 4 < 0.1-4	80	–	–	Hampson et al., 2006
B. intermedia	pig	USA	2008-2010	10	AD	– 0.03 0.008-0.03	0	– 64 1-64	–	–	–	–	–	– 0.25 0.125-0.25	–	– 0.25 0.125-0.25	–	Clothier et al., 2011
B. intermedia	chickens	Belgium, The Netherlands	2008-2010	20	BD	–	–	0.5 8 0.125-8	5	–	–	1 2 0.5- = > 128	10	0.125 0.5 0.031-2	30	0.063 0.25 = > 0.016-0.25	20	Verdillin et al., 2011
B. murdochii	pig	USA	2008-2010	20	AD	– 0.03 0.004-0.03	0	– 64 16-4	100	–	–	–	–	– 1, 0.125-4	–	– 2, 0.125-4	–	Clothier et al., 2011
B. murdochii	pig	USA	2009-2014	24	BD* AD	0.004, 0.08, 0.002-0.016	0	16 32 < = 0.5- > 64	85	–	–	> 128, > 128, < = 2- > 128	80	0.25 2 < = 0.063- > 8	45	0.125 0.5 < = 0.031- > 4	50	Mirajkar et al., 2016
B. murdochii	pigs	Taiwan	Recent	11	AD	0.25 0.25 < = 0.125-0.5	0	16 128 2- > 256	100	–	–	> 256 > 256 8- > 256	81.8	4 8 0.5-64	100	–	–	Yeh et al., 2018
B. innocens	pig	USA	2008-2010	3	AD	– 0.03 0.008-0.015	0	– 64 8-64	100	–	–	–	–	– 0.125 0.125	–	– 0.125 0.125	–	Clothier et al., 2011
B. hampsonii	pig	USA	2009-2014	40	BD* AD	0.004 0.016 0.004-0.25	0	< = 0.5 16 < = 0.5-32	37.5	–	–	4 > 128 < = 2- > 128	25	< = 0.063 0.25 < = 0.063-0.25	10	< = 0.031 < = 0.031 < = 0.031-0.125	0	Mirajkar et al., 2016
B. species	pig	Sweden	1990-1999	16	BD	0.016 0.03 0.008-0.06	0	–	–	–	–	8 1 < = 2-256	6.25	0.06 2 0.03- > 2	25	–	–	Karlsson et al., 2003
B. species	dog	Japan	1999-2000	29	AD	– < 0.006 < 0.006	0	– 1.56 0.39-1.56	0	–	–	– 3.13 0.78-3.13	0	– 6.25 0.2-12.5	–	–	–	Prapasarakul et al., 2003
B. species	pig	USA	2008-2010	11	AD	– 0.03 0.004-0.03	0	– 64 2-64	100	–	–	–	–	– 2 0.125-0.5	–	– 0.5 0.125-0.5	–	Clothier et al., 2011
B. species	chicken, wild mallards	Sweden	2000-2002	48	BD*	–	–	0.5 1 < = 0.5-16	8.3	–	–	2 4 < = 2-8	0	0.125 0.5 < = 0.063-0.5	10.4	0.063 0.25 < = 0.031-.5	18.8	Jansson and Pringle, 2011

(*continued on next page*)

Table 5 (*continued*)

Bacterial strains	host	Country	Bacterial isolating year	Number of isolates	method for AST[a]	carbadox MIC$_{50}$ MIC$_{90}$ Range	% non-wild type[b]	lincomycin MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	tyvalosin MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	tylosin MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	tiamulin MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	valnemulin MIC$_{50}$ MIC$_{90}$ Range	% non-wild type	Reference
B. species	farmed wild boar	Spain	2009-2015	3	BD*	–	–	– – 4-8	66.7	– – 4-8	100	– – 8-32	33.3	– – 1	100	– – 0.25-1	100	Vadillo et al., 2017
B. species	chickens	Germany	2009-2013	37	BD	–	–	= > 16 = > 64 0.25- = > 64	91.2	–	–	= > 128 = > 128 1- = > 128	97.3	1.0 8 0.031- = > 16	73	0.05 4 0.008- = > 4	67.6	Herbst et al., 2018

[a] BD, broth dilution. AD, agar dilution. Studies using the commercial VetMIC Brachy panel are marked with an asterisk*.

[b] The percentage recorded as non-wild type to each antimicrobial was determined using the epidemiological (wild type) cut-off values described by Pringle et al. (2012).

MICs to tiamulin has (Hidalgo et al., 2010; Rugna et al., 2015). This spread is believed to be associated with the movement of pigs that are sub-clinically colonised, particularly pigs transferred from breeding herds to production herds (Hampson et al., 2015). *B. hyodysenteriae* sequence types (STs) or “clones” that include isolates with or without increased MICs to tiamulin are shown in Table 6. This emphasises that independent evolution of increased MIC values to tiamulin occurs amongst isolates within clonal groups. Generally, there has been a trend for isolates in these STs to show increases in MIC against tiamulin with time, although paradoxically German ST122 isolates have shown a trend for a reduction in MICs to tiamulin over time (MIC$_{50}$ shifted from 4 to 2 μg/ml) (Joerling et al., 2018).

5.3. *B. hyodysenteriae strains with high MICs to multiple drugs*

A *B. hyodysenteriae* strain with high MICs to tiamulin, lincomycin, tylosin, doxycycline, and tylvalosin was first reported in The Netherlands in 2008, with valnemulin remaining the only drug registered for the treatment of SD that was still considered to be clinically effective against this strain (Duinhof et al., 2008). Widespread multidrug resistance, including clinical resistance to valnemulin (≥16 μg/ml) was noted in *B. hyodysenteriae* isolates from the Czech Republic around the same time (Lobová et al., 2004), and strains that are resistant to multiple drugs also have been reported in Australia (La et al., 2016) and Italy (Massacci et al., 2018), thus emphasising the global nature of the problem. Antimicrobial usage drives the selection for non-wild type populations, and, consistent with this, increased resistance to pleuromutilins occurred in Czech pig farms after pleuromutilon prescriptions increased following bans on using nitroimidazoles for treating SD and on using olaquindox as a growth promoter (Lobová et al., 2004). Increases in the MIC of several antimicrobials used to treat SD in the USA were largely driven by the emergence of a novel clonal lineage that would later be identified as the new species *Brachyspira hampsonii* (Clothier et al., 2011). However, it was concluded that the rate of evolution from the wild type was not developing as rapidly as seen in isolates from other countries, which could be related to the historic use of carbadox as an in-feed control agent in the USA (Mirajkar et al., 2016). Of the main species of pathogenic intestinal spirochaete identified in this US study, *B. hampsonii* was most likely to have high MICs to multiple drugs.

5.4. *MIC data for B. hyodysenteriae*

MIC data for *B. hyodysenteriae* recovered from pigs in 24 studies from different countries and regions published in the period 2003–2018 are summarised in Table 3. Most studies were from European countries, followed by Far East Asia (Japan, Korea, Taiwan), Australia, the USA and Brazil. One immediate observation is the relative paucity of these reports, which likely reflects the lack of specialised reference laboratories for these important pathogens, and the difficulty faced by routine veterinary diagnostic laboratories in isolating and undertaking MIC testing for the slow-growing anaerobic spirochaetes. The lack of available published data from Southeast Asia and from mainland China, where over half the pigs in the world are located, represents an important gap in global knowledge.

The percentage of isolates recorded as non-wild type in the tables was determined using the ECOFF values described by Pringle et al. (2012) (Table 2). Only five studies examined carbadox: MICs were below 0.25 μg/ml, and no ECOFF values are available. For lincomycin, a high proportion of isolates were non-wild type in most studies (58.6%–100%), and consequently there was no strong temporal trend of increased MICs to lincomycin as MICs were already high in the early 2000s. There were only seven studies that included tyvalosin, and between 66.7% and 95.5% of isolates were non-wild type in these, again with no strong temporal trends for increasing MIC values. For tylosin, non-wild type rates varied from 58.3% in Sweden among isolates from

Table 6
Multilocus sequence types (STs) of *B. hyodysenteriae* containing isolates both with or without increased MICs to tiamulin.

ST	Country	Year of isolation	Publications
ST8	Italy, UK	unknown	Karlsson et al., 2004a; La et al., 2009; Rugna et al., 2015
ST19	Australia	2005	La et al., 2009**
ST21	Australia	2005	La et al., 2009**
ST50	Australia	2014-2016	La et al., 2016
ST51	Germany	1990s	Karlsson et al., 2004a; La et al., 2009
ST52	Italy, Belgium, Germany	1990-2016	Karlsson et al., 2004a; La et al., 2009; Hidalgo et al., 2010; Rugna et al., 2015; Joerling et al., 2018
ST73	Spain	2006, 2007	Hidalgo et al., 2010
ST74	Italy, Sweden	2011	Rugna et al., 2015
ST75	Italy	2003, 2011	Rugna et al., 2015
ST76	Italy	2005-2007, 2011-2012	Rugna et al., 2015
ST77	Italy	2005-2006, 2008, 2010-2012	Rugna et al., 2015
ST78	Italy	2006, 2011	Rugna et al., 2015
ST79	Italy, Belgium, Germany	2007, 2009-2011	Rugna et al., 2015
ST83	Italy	2011-2012	Rugna et al., 2015
ST85	Italy	2011	Rugna et al., 2015
ST86	Italy	2006	Rugna et al., 2015
ST94*	USA	2016	Mirajkar et al., 2014; Mirajkar et al., 2016
ST98	Italy	2007	Rugna et al., 2015
ST103	Italy	2012	Rugna et al., 2015
ST107*	USA	2016	Mirajkar et al., 2014; Mirajkar et al., 2016
ST150	Australia	2014-2016	La et al., 2016

* ST94 and ST107 associated with tiamulin resistance [(ST94: odds ratio (95% CI interval) = ∞ (2.14 to ∞)], [ST107: odds ratio (95% CI interval) = 18.01 (1.37–1071.96)] (Mirajkar et al., 2016).

** La et al., 2009. Australian isolates with clinical breakpoints set at > 4 µg/ml; all other studies cited with clinical breakpoint > 2 µg/ml.

the 1990s (a retrospective study), up to 100% in later studies. The non-wild type rates were very high in most studies, although variation was seen in some locations (for example only 63.3% of isolates in Switzerland in 2009–2015, and 69.6% in Australia in 2014–2016). Interestingly, the non-wild type rate for tylosin was 84.3% in a second more recent Swiss study of isolates from 2010 to 2017 (García-Martín et al., 2018). For tiamulin the rates for non-wild type varied much more, from 0 to 100%. The lowest rates were found in Switzerland (0% in two studies) and in Sweden, South Korea and the USA (25%). In Japan non-wild type rates among isolates increased from 20.8% (1985–2000) to 96.6% (2009). For valnemulin the non-wild type rates among isolates varied from 4.8% in the 1990s in Sweden to 100% in the Czech Republic in 2000-2015. In most studies where isolates from the same region from different time periods were tested these showed an increase in MIC with time for all antimicrobials (e.g. Prášek et al., 2014).

5.5. MIC data for B. pilosicoli

MIC data for *B. pilosicoli* in seven studies, including isolates from pigs and chickens, are shown in Table 4. The trends seen with *B. hyodysenteriae* also were observed with *B. pilosicoli.* MICs for carbadox were below 0.5 µg/ml, and no ECOFF values are available. The non-wild type percentages for lincomycin varied from 58.8% among isolates from the 1990s to 100% in a recent study from Taiwan (Yeh et al., 2018), and similar changes were seen for tylosin. Non-wild type percentages for both pleuromutilins tended to be lower than those seen with *B. hyodysenteriae*, with the highest rate being recorded for valnemulin (58.8%) among isolates from the USA from the period 2009-2014.

5.6. MIC data for other Brachyspira species

MIC data from 15 studies that included *Brachyspira* species other than *B. hyodysenteriae* and *B. pilosicoli* are presented in Table 5. Four studies reported on findings for *B. intermedia* (two using isolates from chickens, and two using isolates from pigs), three studies focused on *B. murdochii* from pigs, one each reported on *B. innocens* and *B. hampsonii* isolates from pigs, and six studies included data for different weakly haemolytic *Brachyspira* species isolated from pigs, dogs, chickens and feral ducks.

Variable percentages of non-wild type isolates were found for all species, but generally *B. murdochii* isolates showed higher percentages of non-wild type isolates than the other species. This needs to be interpreted with caution, as there is a lack of validated epidemiological cut of values for species other than *B. hyodysenteriae*. Recent unidentified *Brachyspira* isolates from Spanish farmed wild boar (Vadillo et al., 2017) and German chickens (Herbst et al., 2018) showed high MICs to the agents tested, despite these animals likely having been subjected to less antimicrobial pressure than other more intensively housed species.

6. Mechanisms involved in increased MIC values to different antimicrobial agents

The mechanisms involved in reduced susceptibility by *Brachyspira* species to different antimicrobial agents have been relatively poorly studied compared to other bacterial groups. Potential mechanisms such as efflux pumps have not been investigated to date. Most of the important antimicrobial agents used to control the infections in pigs and poultry inhibit protein synthesis by binding to the 23S rRNA of the 50S subunit of the ribosome, and resistance is associated with single nucleotide polymorphisms (SNPs) in the rRNA gene sequences and/or mutations in the genes encoding ribosomal proteins L2, L3, L4 and L22 that result in reduced binding of the antimicrobials to the target sites. At this time a clear and widely agreed association between specific mutational changes at different ribosomal gene sites and increased MIC values to different antimicrobials in *Brachyspira* species is only available in a few cases. The most commonly described genetic changes and genes associated with antimicrobial resistance to the main therapeutic drugs in *B. hyodysenteriae* are summarised in Table 7. Other infrequently reported SNPs in some resistant isolates may contribute to reduced antimicrobial binding, but the evidence is less compelling than for the SNPs that are present in most resistant isolates. A more comprehensive description of reported resistance mechanism is presented below.

6.1. Macrolides and lincosamides

A mechanism causing resistance to tylosin among *B. hyodysenteriae* isolates was first identified by Karlsson et al (1999), who associated resistance with a mutation in the nucleotide position homologous with

Table 7
Some commonly described genetic changes associated with reduced susceptibility to antimicrobial drugs in *Brachyspira hyodysenteriae*[*].

Antimicrobials	SNPs in the 23S rRNA gene	Alterations in L3 ribosomal proteins	SNPs in the 16S rRNA gene	Genes
Macrolides and lincosamides	A2058T (Karlsson et al., 1999) A2059 G; A2059C (Karlsson et al., 2004b) A2058G (Hidalgo et al., 2011)	–	–	*lnu*(C) [lincomycin resistance] (De Luca et al., 2018)
Pleuromutilins	G2032A (Pringle et al., 2004)	Asn148Ser (Pringle et al., 2004)	–	*tva*(A) (Card et al., 2018)
Doxycycline	–	–	G1058C (Pringle et al., 2007) G1058T (Card et al., 2018)	–

[*] Some *B. pilosicoli* isolates have $bla_{\text{OXA-63}}$ group genes encoding Class D β-lactamases that inactivate and hence cause resistance to ampicillin and oxacillin.

position 2058 of the *Escherichia coli* 23S rRNA gene, where A was substituted for T (A2058 T). This same transversion is a cause of macrolide resistance in other bacterial species, where it is associated with reduced macrolide binding. Subsequently this transversion, as well as an A2059 G transition and an A2095C transversion that caused macrolide and lincosamide resistance were identified in the 23S rRNA gene of resistant *B. pilosicoli* isolates (Karlsson et al., 2004b). In a later study, decreased susceptibility to tylosin and lincomycin in *B. hyodysenteriae* isolates was associated with either the A2058 T transversion or less frequently an A2058 G transition (Hildago et al., 2011). In addition, G2057 A plus C2611 T transitions at the base pairs adjacent to A2058 G were identified in three isolates, and it was suggested that in some way these might reduce the fitness cost of the A2058 G transition. The A2058 T transversion was also found in Belgian *B. hyodysenteriae* isolates that were resistant to lincomycin, tylosin and tylvalosin, although T2528C and G2535 A mutations also were found in some resistant isolates (Mahu et al., 2017). The relevance of the latter changes were not clear as it is not known whether and to what extent they might result in reduced drug binding. The A2058 T transversion also has been identified in Korean *B. hyodysenteriae* isolates that were resistant to macrolides and lincomycin (Yeh et al., 2018)

Additional mechanisms contributing to resistance to lincomycin recently have been recognised. A nucleotidyltransferase gene *lnu*(C) was found on a small 1724-bp transposon in an Italian multidrug-resistant strain of *B. hyodysenteriae* (BH718) that was subjected to genome sequencing (De Luca et al., 2018). This gene encodes an enzyme that inactivates lincosamides, and hence confers resistance to lincomycin. A blast search identified the same transposon (MTnSag1) in Australian *B. pilosicoli* strain 95/1000 isolated from a pig. BH718 had the A2058 G mutation in the 23S rRNA gene that confers resistance to macrolides and lincosamides, and genome sequencing of other resistant *B. hyodysenteriae* strains confirmed a strong correlation between this and decreased susceptibility to tylosin and lincomycin. However, strains belonging to sequence type 83 (ST83), which includes BH718, and that also possessed *lnu*(C), had an even greater reduction in susceptibility to lincomycin than strains in other STs that had the mutations but not the gene. This finding strongly suggests the involvement of *lnu*(C) in contributing to reduced susceptibility to lincomycin. Further studies are now required to investigate the presence of this transposon and the *lnu* (C) gene in other *B. hyodysenteriae* strains and in other *Brachyspira* species.

6.2. Pleuromutilins

Like the macrolides and lincosamides, the pleuromutilins target the large subunit of the bacterial ribosome where they act to inhibit protein synthesis. Increases in MICs to tiamulin can be induced *in vitro* in a step-wise fashion following multiple sub-cultures of *Brachyspira* spp. on agar that contains increasing concentrations of the antimicrobial (Karlsson et al., 2001). Interestingly, some *B. pilosicoli* cells have been shown to survive even very high concentrations of tiamulin, which may contribute to disease re-emergence after antibiotic treatment (Le Roy et al., 2017). Pringle et al. (2004) identified putative tiamulin resistance mechanisms by genetically characterising laboratory-induced mutants, field strains and tiamulin-resistant *B. hyodysenteriae* strains. Resistance in laboratory mutants appeared to be related to reduction of tiamulin binding to the ribosomal peptidyl transferase centre (PTC) through one or more point mutations in the genes encoding ribosomal protein L3 and 23S rRNA at positions proximal to the PTC. Different resistant isolates had mutations G2032 A, C2055 A, G2447 T, C2499 A, T2504 G or A2572Y in the 23S rRNA (*E. coli* numbering), and/or had ribosomal protein L3 mutations Asn148Ser, Asn148Lys, or Ser149[mixed sequence] (*B. pilosicoli* numbering). In chemical footprinting experiments mutants with reduced susceptibility to tiamulin were shown to have reduced binding of the drug to ribosomal subunits. In a later study of tiamulin-resistant Spanish *B. hyodysenteriae* isolates a G2032 A transition also was commonly found (Hidalgo et al., 2011). In German *B. hyodysenteriae* field strains, resistance to pleuromutilins again was strongly associated with single point mutations in the ribosomal protein L3 gene leading to a Asn148Ser substitution (Hillen et al., 2014). In this study a total of 24 single nucleotide mutations in the genes encoding ribosomal proteins L2, L3, L4, L22, and in the 23S rRNA gene in the peptidyl transferase region were found in different isolates. Amongst 18 Belgian *B. hyodysenteriae* isolates showing evidence of acquired resistance to pleuromutilins, 16 showed an A2058 T transversion, although this was also found in some susceptible strains (Mahu et al., 2017). In four of the 18 resistant isolates there was an Asn148Ser mutation for the L3 protein. The inconsistent nature of these changes found in some of the resistant isolates makes it difficult to assess the extent that each may have in contributing to acquired resistance.

Recently a newly described gene designated *tva*(A) that contributes to pleuromutilin resistance in *B. hyodysenteriae* was identified following whole genome sequencing of resistant and control strains, where genome-wide association studies linked the presence of *tva*(A) with tiamulin resistance (Card et al., 2018). This gene encodes a predicted ABC-F transporter that may act as a ribosomal protection protein: although this confers reduced pleuromutilin susceptibility it does not by itself result in clinical resistance. When the gene is present in strains with the previously described mutations in ribosomal genes it leads to higher level of resistance than where the mutations occur in the absence of *tva*(A). In farms with SD where pleuromutilins are being used for control of the disease, the presence of *tva*(A) in *B. hyodysenteriae* strains is predicted to broaden the mutant selection window and raise the recommended concentration of tiamulin that should be used to prevent selection of resistant mutations. This study also identified new mutations in the 23S rRNA gene and in the gene encoding the L3 ribosomal protein that potentially may be associated with reduced susceptibility to tiamulin. Mutations in the Elongation Factor G (EF-G) gene (*fusA*) also were identified in three resistant strains. EF-G is involved in the translocation step of the elongation cycle of bacterial protein synthesis, directly following the peptide-bond formation step which is inhibited by pleuromutilins (Card et al., 2018). Hence these mutations potentially also may have some role in reducing susceptibility to tiamulin. Studies into the distribution of *tva*(A) in strains of *B. hyodysenteriae* and other *Brachyspira* species are needed, and investigations into the significance of the newly described mutations are required.

6.3. Doxycycline

Doxycyclin is not widely used for the control of SD. Nevertheless, resistance to doxycycline in *B. hyodysenteriae* has been associated with a single nucleotide transversion at position 1058 of the 16S rRNA gene of the 30S ribosomal subunit (G1058C), a position known to be associated with doxycycline resistance in other bacterial species (Pringle et al., 2007). This same transversion subsequently was confirmed in doxycycline-resistant *B. intermedia* isolates from chickens (Verlinden et al., 2011), and in doxycycline-resistant *B. hyodysenteriae* isolates from pigs in Switzerland (García-Martín et al., 2018) and Belgium (Mahu et al., 2017). Another transversion (G1058 T) recently was identified in the *B. hyodysenteriae*16S rRNA gene that was associated with reduced susceptibility to doxycycline in some isolates (Card et al., 2018).

6.4. Penicillins

Another resistance mechanism found in *B. pilosicoli* is the presence of specific genes encoding enzymes that inactivate penicillins. Many strains of *B. pilosicoli* from diverse geographical regions and host species have been shown to harbour novel $bla_{\text{OXA-63}}$ group genes encoding Class D β-lactamases. To date 14 different variants of this gene have been identified that encode β-lactamases that inactivate ampicillin and oxacillin (OXA-63, OXA-136, OXA-137, and OXA-470 through OXA-479), and these do not appear to be present in other *Brachyspira* species (La et al., 2015). From a clinical perspective their main significance is in humans colonised with *B. pilosicoli,* where penicillins are sometimes used for treatment of clinical signs and to clear chronic colonisation.

7. Future control, including use of genomics in surveillance

A major constraint to understanding the extent of the emergence of antimicrobial resistance in *Brachyspira* species has been a lack of standardised testing methods and established breakpoints, so that results from different studies sometimes are difficult to interpret. These methods and breakpoints need to be developed and applied in a carefully planned and systematic way to determine and monitor the situation for the various *Brachyspira* species in different regions of the world.

The recent availability of rapid and inexpensive whole genome sequencing is facilitating genome wide association studies using wild-type and non-wild type strains to permit a deeper understanding of potential mechanisms of resistance to different antimicrobials. Once mechanisms are understood, resistance markers can be validated and used in diagnostic and research settings. In particular, whole genome sequencing of individual isolates allows them to be assigned to a clonal group, given a virulence gene profile, and annotated as possessing potential SNPs or genes predicted to encode resistance to important antimicrobial agents. These data can be stored in databases and used to aid the monitoring of resistance both on-farm and within reference laboratories. Better surveillance and detection of virulent and resistant strains also will aid biosecurity efforts intended to reduce the introduction of these strains to naïve farms or farms that do not harbour problematic strains. Beyond identifying resistance mechanisms and occurrence of resistance, future work will continue to investigate other means to control infections with *Brachyspira* species, most likely through the development of new and effective vaccines, and using diet or specialised nutritional supplements to modulate the physicochemical and microbial environment in the large intestine in a way that reduces *Brachyspira* growth and survival.

8. Conclusions

The genus *Brachyspira* includes a number of important pathogenic species for which the main means of control has been either prophylactic or therapeutic application of antimicrobial agents. In this mini-review we have outlined how the MICs of the main antimicrobial agents have increased with time, to the extent that clinical resistance to most of the previously effective agents is now common amongst the different pathogenic *Brachyspira* species. Consequently, it is important to determine MIC values before starting treatments, and where an MIC remains low the antimicrobial should be used to try to eradicate the infection, rather than simply applying it for disease control.

Conflict of interest

None of the authors have financial or personal relationships with people or organisations that could represent a conflict of interest.

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