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Antimicrobial sensitivity testing of Australian isolates of *Bordetella avium* and the *Bordetella avium*-like organism

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SUMMARY: The in-vitro sensitivity of 16 Australian isolates of *Bordetella avium* and 15 isolates of *B avium*-like organism to 11 antimicrobial agents or combinations of agents was determined using a microtitre plate system to establish minimal inhibitory concentrations. All the *B avium* isolates were sensitive to ampicillin but resistant to erythromycin, lincomycin, spectinomycin, sulphamethoxazole, trimethoprim, and lincomycin + spectinomycin. Most of the *B avium* isolates were sensitive to tetracycline and resistant to streptomycin and sulphadiazine. All the *B avium*-like isolates were resistant to ampicillin, erythromycin, lincomycin, spectinomycin, streptomycin, tetracycline, trimethoprim, and lincomycin + spectinomycin. Most *B avium*-like isolates were sensitive to sulphadiazine, sulphamethoxazole and trimethoprim-sulphamethoxazole.
Aust Vet J **72**: 97 – 100

Introduction

Bordetella avium is recognised as the aetiological agent of a specific respiratory disease of turkeys called turkey coryza (Arp and Skeeles 1991). The disease is characterised by snicking, ocular discharge, conjunctivitis, decreased appetite and weight gain, depression, dyspnoea and death (Arp and Skeeles 1991).

Following the first reported isolation of *B avium* from Australian chickens and turkeys (Blackall and Farrah 1985), we have conducted a series of investigations to characterise Australian isolates of *B avium* and related species. We have established that both *B avium* and a related organism called the *B avium*-like organism are present in Australian poultry (Blackall and Doheny 1987). A review of the available case histories suggested an association between *B avium* and upper respiratory tract disease in Australian turkeys (Blackall and Doheny 1987). In contrast, there was no such association between the *B avium*-like organism and respiratory disease in chickens or turkeys (Blackall and Doheny 1987). We have established several alternative methods for identifying these organisms – alkalinisation patterns (Blackall and Doheny 1987), use of the API20NE, a commercial microidentification kit (Blackall and Doheny 1987) and cellular fatty acid profiles as detected by gas chromatography (Moore *et al* 1987).

TABLE 1

Minimal inhibitory concentrations (MIC) of 11 antimicrobial agents or combinations of agents against 16 *B avium* isolates

Antimicrobial agent	No. of isolates with indicated MIC (μg/mL)												
	> 128	128	64	32	16	8	4	2	1	0.5	0.25	0.125	< 0.125
Ampicillin	-	-	-	-	-	-	-	-	1	10	5	-	-
Erythromycin	-	-	-	-	1	15	-	-	-	-	-	-	-
Lincomycin	16	-	-	-	-	-	-	-	-	-	-	-	-
Spectinomycin	-	1	15	-	-	-	-	-	-	-	-	-	-
Streptomycin	-	-	-	-	15	1	-	-	-	-	-	-	-
Sulphadiazine	15	-	-	1	-	-	-	-	-	-	-	-	-
Sulphamethoxazole	16	-	-	-	-	-	-	-	-	-	-	-	-
Tetracycline	1	-	-	1	-	-	2	2	10	-	-	-	-
Trimethoprim	1	-	2	2	11	-	-	-	-	-	-	-	-
Lincomycin + spectinomycin	-	16	-	-	-	-	-	-	-	-	-	-	-
Trimethoprim + sulphamethoxazole	5	1	2	1	7	-	-	-	-	-	-	-	-

TABLE 2

Minimal inhibitory concentrations (MIC) of 11 antimicrobial agents or combinations of agents against 15 *B avium*-like isolates

Antimicrobial agent	No. of isolates with indicated MIC (μg/mL)												
	> 128	128	64	32	16	8	4	2	1	0.5	0.25	0.125	< 0.125
Ampicillin	-	-	1	3	11	-	-	-	-	-	-	-	-
Erythromycin	-	-	-	-	14	1	-	-	-	-	-	-	-
Lincomycin	15	-	-	-	-	-	-	-	-	-	-	-	-
Spectinomycin	15	-	-	-	-	-	-	-	-	-	-	-	-
Streptomycin	-	-	-	1	13	1	-	-	-	-	-	-	-
Sulphadiazine	2	-	-	-	-	10	3	-	-	-	-	-	-
Sulphamethoxazole	2	-	-	-	-	6	7	-	-	-	-	-	-
Tetracycline	1	-	-	1	2	1	7	3	-	-	-	-	-
Trimethoprim	-	-	4	11	-	-	-	-	-	-	-	-	-
Lincomycin + spectinomycin	15	-	-	-	-	-	-	-	-	-	-	-	-
Trimethoprim + sulphamethoxazole	2	-	-	-	-	4	3	4	2	-	-	-	-

As part of the basic characterisation of Australian isolates of *B avium* and the *B avium*-like organism, we report here the results of in-vitro antimicrobial sensitivity testing using a microtitre plate system to determine the minimum inhibitory concentration (MIC) of a range of antimicrobial agents. This was done to provide information on the antimicrobial agents likely to be of use in the empirical treatment of outbreaks of turkey coryza in Australian turkeys.

Materials and Methods

Bacteria

The 16 *B avium* and 15 *B avium*-like isolates used in this study were identified by substrate alkalinisation patterns, API20NE profile and fatty acid profiles as described previously (Blackall and Doheny 1987; Moore *et al* 1987).

* Oxoid CM473, Oxoid Australia Pty Ltd, West Heidelberg, Vic

Antimicrobial Sensitivity Testing

Solutions of the following antimicrobial agents were prepared as described by Anhalt and Washington (1985): ampicillin, erythromycin, lincomycin, spectinomycin, streptomycin, sulphadiazine, sulphamethoxazole, tetracycline and trimethoprim. Combinations of agents were prepared in the following ratios: lincomycin-spectinomycin (1:2), trimethoprim-sulphamethoxazole (1:19).

Doubling dilutions of the antimicrobial agents were prepared in Isosensitest broth* (ISB) using the technique described by Waterworth (1978a). The various dilutions of antimicrobial agents containing from 256 to 0.25 μg/mL were dispensed in 50 μL volumes sterile, tissue-culture quality, 96-well U-bottomed microtitration plates such that well 1 of each row contained the agent at 256 μg/mL and well 11 contained the agent at 0.25 μg/mL. Well 12 of each row was a control well containing only ISB. Overnight cultures of test organisms, grown on blood agar, were inoculated into 2 mL of ISB

and incubated for 6 h. Plate counts on a range of *B avium* and *B avium*-like organisms had shown such broths to contain an average of 1×10^8 colony forming units (CFU)/mL. The cultures were diluted 1/1000 to provide an estimated 1×10^5 CFU/mL in ISB and 50 µL of this adjusted suspension added to each well.

The inoculated plates were placed in a sealed container and incubated under aerobic conditions at 37°C for 24 h. The MIC was taken as the lowest concentration of an antimicrobial agent to completely prevent growth as determined visually. When testing sulphonamide agents, including the trimethoprim-sulphamethoxazole combination, and trimethoprim, the dilutions of the agents were prepared using 8% (v/v) lysed horse blood, yielding a final concentration of 4% lysed horse blood. The lysed horse blood was used to block the action of any antagonists (Waterworth 1978b). As the volume of the inoculum was equal to the volume of medium in each well, the possible MIC values ranged from > 128 µg/mL (growth in wells 1 to 12) to < 0.125 µg/mL (growth only in well 12). The purity and viability of each culture was confirmed by inoculation onto a blood agar plate. A reference *Escherichia coli* strain (ATCC 25922) was included with each set of tests performed.

Results

The MICs of the 11 antimicrobial agents or combinations of agents are shown in Tables 1 (*B avium*) and 2 (*B avium*-like organism). For both taxa, the MICs fell within a narrow range for ampicillin, erythromycin, lincomycin, spectinomycin, streptomycin and lincomycin-streptomycin. However, for sulphadiazine, sulphamethoxazole, tetracycline,trimethoprimand trimethoprim-sulphamethoxazole, a wider range of MICs was encountered. The MIC values determined for the reference *E coli* strain (ATCC 25922) were reproducible within one doubling dilution and fell within the range given by Jones *et al* (1985) for this organism.

To allow the MIC results to be interpreted as "sensitive" or "resistant", a breakpoint for each antimicrobial agent must be determined. The breakpoint is defined as that MIC that separates sensitive from resistant and is ideally based on obtainable serum or tissue concentrations of the antimicrobial agent (Libal *et al* 1986). The breakpoints used in this study are shown in Table 3. The breakpoints were as provided by Libal *et al* (1986) or, if necessary, were obtained from other sources (Garrod *et al* 1973; Baggot 1978; Hooke 1978; Wise 1978; Fales *et al* 1986; Anonymous 1988). The percentage of sensitive *B avium* and *B avium*-like isolates is shown in Table 3. The only antimicrobial agent to which all *B avium* isolates were sensitive was ampicillin. In contrast, all *B avium*-like isolates were resistant to this agent. The only other antimicrobial agents to which a significant number of *B avium* isolates were sensitive were tetracycline and trimethoprim-sulphamethoxazole. Most *B avium*-like isolates were sensitive to the sulphonamides and trimethoprim-sulphamethoxazole.

Discussion

The in-vitro antimicrobial sensitivity patterns of *B avium* and the *B avium*-like organism have not received much attention. Previous studies have been performed mainly by disc diffusion techniques (Simmons *et al* 1980; Luginbuhl *et al* 1984, 1986; Jackwood *et al* 1987). In the only MIC study to date, Mortensen *et al* (1989) examined 10 *B avium* isolates using a commercial system†. As this commercial system was developed for use in human medicine, many of the antimicrobial agents examined by Mortensen *et al* (1989) are not relevant to veterinary medicine. Our study represents the first report of the MICs of a range of relevant antimicrobial agents for both *B avium* and the *B avium*-like organism.

† Beckman Panels, Beckman Instruments, Fullerton, CA

TABLE 3
Breakpoint minimal inhibitory concentrations (MIC)* of 11 antimicrobial agents or combinations of agents and the percentage of sensitive organisms for 16 *B avium* isolates and 15 *B avium*-like isolates

Antimicrobial agent	MIC breakpoint (µg/mL)	*B avium* (Percent (sensitive)	*B avium*-like (Percent sensitive)
Ampicillin	≥ 2	100	0
Erythromycin	≥ 1	0	0
Lincomycin	≥ 16	0	0
Spectinomycin	≥ 16	0	0
Streptomycin	≥ 16	6.3	0
Sulphadiazine	≥ 128	6.3	86.7
Sulphamethoxazole	≥ 128	0	86.7
Tetracycline	≥ 2	62.5	0
Trimethoprim	≥ 1	0	0
Lincomycin + spectinomycin	≥ 16	0	0
Trimethoprim + sulphamethoxazole	≥ 32	43.8	86.7

* The breakpoint MIC is defined as that MIC that separates sensitive from resistant and is ideally based on obtainable serum or tissue concentrations of the antimicrobial agent (Libal *et al* 1986).

Only four antimicrobial agents, ampicillin, erythromycin, tetracycline and trimethoprim-sulphamethoxazole, were examined in both our study and that of Mortensen *et al* (1989). The MICs for these agents were in agreement in the two studies except that our MIC range for trimethoprim-sulphamethoxazole (8- ≥ 128 µg/mL) was higher than that reported by Mortensen *et al* (1989) (≤ 0.5-16 µg/mL). This difference may be explained by the fact that all the isolates we used were from Australian poultry whereas Mortensen *et al* (1989) used isolates from American poultry.

To enable an interpretation of the MIC results, we have chosen to use the breakpoint definition of Libal *et al* (1986). Under this definition, when the MIC of an antimicrobial agent for an isolate equals or exceeds the breakpoint the isolate is regarded as resistant to that agent (Libal *et al* 1986). Ideally, the breakpoint is based on the highest concentration of the agent obtainable in serum or tissue. We have been unable to locate specific data for poultry and have used breakpoints published for food animals in general. The problem of a lack of agreed guidelines for any animal species for the interpretation of MIC data has been noted by Prescott and Baggot (1988).

In common with the studies of Luginbuhl *et al* (1986) and Jackwood *et al* (1987) we found a high prevalence of resistance, in both *B avium* and the *B avium*-like organism, to streptomycin and tetracycline. Like Luginbuhl *et al* (1986), we found that *B avium* isolates were sensitive to ampicillin and mainly resistant to the sulphonamides.

The wide ranges of the MICs of sulphadiazine and tetracycline for both *B avium* and the *B avium*-like organism indicate the existence of acquired resistance. Luginbuhl *et al* (1986) and Jackwood *et al* (1987) have correlated the presence of plasmids with resistance to these antimicrobial agents.

Our finding that *B avium* isolates are uniformly sensitive to ampicillin whereas *B avium*-like isolates are uniformly resistant has been proposed previously as an aid in the separation of these two taxa (Tillack and Blackall 1994). Resistance, in gram negative bacteria, to beta-lactam antibiotics such as ampicillin is associated

principally with three cellular components, which can act individually or in conjunction. These components are (a) a set of channel-forming outer membrane proteins, called porins, through which the beta-lactam antibiotics diffuse (Nikaido 1976), (b) the targets of beta-lactam antibiotic action, the so-called penicillin-binding proteins (Spratt 1977) and (c) beta-lactamases, enzymes that inactivate beta-lactams by various mechanisms (Collatz *et al* 1984). The cellular component or components responsible for the ampicillin resistance of the *B avium*-like isolates remains to be established.

The effectiveness of antimicrobial agents in the treatment of turkey coryza remains a contentious issue despite a series of studies performed in other countries. Glunder *et al* (1979) found that treatment of infected 7-week-old poults with combinations of tetracycline and sulphaquinoxaline/trimethoprim reduced the incidence of clinical disease during treatment but that the signs reappeared once the treatment ceased. As well, *B avium* could be isolated from the treated birds. Skeeles *et al* (1983) found that an injectable long-acting tetracycline had no obvious effect upon either clinical signs or the presence of the organism. In contrast, in mature turkey breeders, Kelly *et al* (1986) found that a penicillin/tetracycline-HCl combination proved very effective in relieving the clinical signs of turkey coryza.

In an attempt to develop effective treatment methods, aerosolisation of a broad-spectrum antibiotic such as tetracycline has been examined in other countries with conflicting results. Ficken (1983) found a significant reduction in clinical signs. Van Alstine and Hofstad (1985) found only a temporary decrease in bacterial colonisation and a delay in clinical signs, with no difference between treated and untreated birds 4 days after the treatment was ceased.

In conclusion, our in-vitro study showed that Australian isolates of *B avium* were resistant to a range of antimicrobial agents. Ampicillin, tetracycline and trimethoprim-sulphamethoxazole showed activity against *B avium*. In practical terms this means that any empirical treatment programmes for turkey coryza outbreaks in Australia should be based on the use of these antibiotics. However, the relatively high levels of resistance to tetracycline (37% of isolates) and trimethoprim-sulphamethoxazole (56% of isolates) indicates that these agents may be of limited use.

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Modified lateral spinal decompression in dogs with thoracolumbar disc protrusion

A modified lateral spinal decompression technique was performed in 61 dogs with thoracolumbar disc protrusion by Yovich *et al* (1994) *J Small Anim Pract* **35**:351-356. Myelography combined with plain radiography and neurological examination determined the side of greatest compression in 93% of the dogs. Disc material was retrieved in 98% of the cases. Of the 35 non-ambulatory dogs, 95% regained the ability to walk. The recovery time was three weeks.