Surveillance for antibiotic resistance in veterinary pathogens from the perspective of a regional diagnostic laboratory

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Abstract

The Toowoomba Veterinary Laboratory tests for antibiotic resistance through passive surveillance of bacterial pathogens from diseased, frequently intensively managed, animals. Testing is carried out on the basis of the number of animals involved, the nature and severity of the disease and the identity and significance of the bacterium, the results guiding the submitting veterinarian in implementing appropriate treatment. The antibiotics chosen for testing are those that are currently registered for veterinary use and are considered effective in the given situation. Testing is carried out according to the current National Committee for Clinical Laboratory Standards Approved Standard for Disc Susceptibility Tests. This paper presents some results of testing bacterial pathogens from cattle and pigs. *Commun Dis Intell* 2003;27 Suppl:S127–S131.

Keywords: antibiotic resistance, veterinary pathogens, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa

Introduction

The Toowoomba Veterinary Laboratory is operated by the Animal and Plant Health Service of the Queensland Department of Primary Industries. The laboratory provides a comprehensive disease investigation and surveillance service for commercial livestock producers throughout South-East Queensland and receives approximately 4,000 accessions per year. Samples may be submitted to the laboratory from either live or dead animals or whole animals may be brought to the laboratory for necropsy. Diagnostic testing of commercial livestock is provided free of charge to primary producers and veterinarians. The diagnostic service provided by the laboratory assists producers to maintain sustainable production levels by implementation of appropriate treatment and control programs. The laboratory maintains a surveillance program for both exotic and endemic disease, helping to ensure market access for animals and animal products and the provision of wholesome animal products to consumers.

As the area serviced by the Toowoomba Veterinary Laboratory contains 45 per cent of the State's cattle and more than half of Queensland's pigs, bacterial pathogens from these animals are of major interest. The laboratory routinely carries out susceptibility testing on bacterial pathogens isolated from diseased tissues. Susceptibility testing of organisms involved in mastitis is also performed.

Methods

The Toowoomba Veterinary Laboratory is accredited for Veterinary Testing by the National Association of Testing Authorities to Australian Standard AS ISO/IEC 17025. The method used for susceptibility testing is that specified by the National Committee for Clinical Laboratory Standards for disc susceptibility tests.^{1,2} Quality control is carried out weekly using the type strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

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Antibiotic susceptibility testing is not carried out on all bacteria isolated from diseased animals. The decision to proceed with susceptibility testing is based on the number and value of animals involved, the nature and severity of the disease and the identity and significance of the bacterium isolated. A susceptibility result is only provided for isolates obtained in pure or almost pure culture that are considered to be primary or significant contributing factors in the disease syndromes under investigation. Susceptibility testing is only carried out on rapidly growing, aerobic bacteria. Antibiotic susceptibility test results are generally provided within 48 hours of receipt of the submission.

The antibiotics chosen for testing reflect those drugs that are currently registered for veterinary use in Australia and are considered most effective by veterinarians. A panel of antibiotics is tested against each of the pathogen groups. While reasonably stable, the composition of these panels may change from time to time according to the registration or de-registration of particular antibiotics and their availability, cost and effectiveness in the field. The Toowoomba Veterinary Laboratory runs between 400 and 450 susceptibility test panels per annum, each consisting of from seven to nine antibiotics.

Currently enteric pathogens are tested against the antibiotics ampicillin, apramycin, ceftiofur, cotrimoxazole, lincospectin, neomycin and tetracycline. Non-enteric pathogens are tested against ampicillin, ceftiofur, cotrimoxazole, lincospectin, neomycin, tetracycline and penicillin. Organisms isolated from cases of mastitis are tested against ampicillin, cefuroxime, clindamycin, cloxacillin, neomycin, novobiocin, penicillin and tetracycline.

Results

Bovine

Mastitis is an ongoing problem in dairy cows, from which the most frequently isolated aetiological agent is *Staphylococcus aureus*. Nine antibiotics, listed above, are routinely tested against bacterial isolates from bovine mastitis (Table 1). Over a three-year period, from 1999 to 2001, one third of isolates were found to be resistant to ampicillin and penicillin, while a small number were resistant to novobiocin. No other resistance was detected.

Table 1. Results of antibiotic susceptibility testing of strains of Staphylococcus aureus isolated from bovine mastitic milk between 1999 and 2001

Antibiotic	AMP10	СХМЗО	DA2	N30	NV30	0B5	P10	TE30
Number of strains tested	121	107	121	121	120	121	121	121
Number of strains resistant	40	0	0	0	3	0	40	0
Strains resistant (%)	33.1	0	0	0	2.5	0	33.1	0

AMP10 = ampicillin (10 μ g); CXM30 = cefuroxime (30 μ g); DA2 = clindamycin 2 μ g); N30 = neomycin (30 μ g); novobiocin (30 μ g); OB5 = cloxacillin (5 μ g); P10 = penicillin (10 i.u.); TE30 = tetracycline (30 μ g).

Scours as a result of non-haemolytic *Escherichia coli* infection is a common problem in young calves. For the purposes of comparison, the results of antibiotic testing of these isolates in 1999 and 2001 in are given in Table 2. Resistance was detected in tetracycline, ampicillin and cotrimoxazole, with levels appearing to increase, particularly against tetracycline. Levels of resistance to neomycin appear stable, while a single isolate demonstrated resistance to apramycin in 2001.

	1999			2001			
	Tested	Resistant	Resistant %	Tested	Resistant	Resistant %	
AMP10	11	4	36.3	18	11	61.1	
APR15	11	0	0.0	18	1	5.6	
EFT30	11	0	0.0	18	0	0.0	
N30	11	6	54.5	18	9	50.0	
SXT25	11	3	27.2	18	8	44.4	
TE30	11	4	36.3	18	14	77.8	

Table 2. Results of antibiotic sensitivity testing of strains of non-haemolytic *Escherichia coli* isolated from the intestine of calves with scours between 1999 and 2001

AMP10 = ampicillin (10 μ g); CXM30 = cefuroxime (30 μ g); DA2 = clindamycin 2 μ g); N30 = neomycin (30 μ g);

novobiocin (30 μ g); OB5 = cloxacillin (5 μ g); P10 = penicillin (10 i.u.); TE30 = tetracycline (30 μ g).

Salmonellosis is also diagnosed in cattle, in calves as well as mature animals. Results of testing between 1999 and 2001, presented in Table 3, reveal that half of the isolates of *Salmonella* tested were resistant to tetracycline, while one isolate was resistant to cotrimoxazole.

Table 3. Results of antibiotic sensitivity testing of strains of Salmonella sp. isolated from the intestine of calves diagnosed with salmonellosis between 1999 to 2001

Antibiotic	AMP10	APR15	EFT30	N30	SXT25	TE30
No. of strains tested	21	22	22	22	22	22
No. of strains resistant	0	0	0	0	1	11
Strains resistant (%)	0	0	0	0	9.1	50

AMP10 = ampicillin (10 μ g); CXM30 = cefuroxime (30 μ g); DA2 = clindamycin 2 μ g); N30 = neomycin (30 μ g); novobiocin (30 μ g); OB5 = cloxacillin (5 μ g); P10 = penicillin (10 i.u.); TE30 = tetracycline (30 μ g).

Porcine

Scours in neo-natal and young animals due to infection with haemolytic *Escherichia coli* is one of the disease syndromes most frequently diagnosed in pigs and one associated with higher levels of antibiotic resistance. Nonetheless, with the exception of tetracycline to which 100 per cent of isolates obtained in 2001 were resistant, levels of resistance do not appear to have increased between 1999 and 2001. These results are given in Table 4.

		1999			2001	
	Tested	Resistant	Resistant %	Tested	Resistant	Resistant %
AMP10	34	12	35.3	34	8	23.5
APR15	34	13	38.2	34	12	35.3
EFT30	34	0	0.0	34	0	0.0
N30	34	12	35.3	34	15	44.1
SXT25	34	21	61.8	34	16	47.1
TE30	34	30	88.2	34	34	100.0

Table 4. Results of antibiotic sensitivity testing of strains of haemolytic *Escherichia coli* isolated fromthe intestine of pigs with enteric disease between 1999 and 2001

Antibiotic susceptibility testing is also carried out against respiratory pathogens isolated from pigs. Results of testing *Pasteurella multocida* isolated from cases of porcine respiratory disease are given in Table 5. While levels of resistance to penicillin and tetracycline rose over this time, resistance to other antibiotics was not detected.

Table 5. Results of antibiotic sensitivity testing of strains of Pasteurella multocida isolated from therespiratory tract of pigs with pneumonia between March 1998 and March 1999 and between March2001 and March 2002

	March 1998 to March 1999			March 2001 to March 2002			
	Tested	Resistant	Resistant %	Tested	Resistant	Resistant %	
AMP10	20	0	0.0	17	0	0.0	
EFT30	20	0	0.0	17	0	0.0	
LS109	20	0	0.0	5	0	0.0	
N30	20	0	0.0	17	0	0.0	
P10	19	3	15.8	16	6	37.5	
SXT25	20	0	0.0	17	0	0.0	
TE30	20	1	5.0	17	4	23.5	

AMP10 = ampicillin (10 μ g); CXM30 = cefuroxime (30 μ g); DA2 = clindamycin 2 μ g); N30 = neomycin (30 μ g); novobiocin (30 μ g); OB5 = cloxacillin (5 μ g); P10 = penicillin (10 i.u.); TE30 = tetracycline (30 μ g).

Discussion

The Toowoomba Veterinary Laboratory provides a veterinary diagnostic and disease surveillance service for commercial animal production in south-east Queensland. An integral component of this activity is the provision of antibiotic susceptibility testing of bacteria isolated from diseased livestock and believed to be significant with respect to the disease under investigation. Providing the results of such testing in a timely manner promotes the judicious and targeted use of antibiotics for the treatment of animal disease. Such treatment is necessary in order to contain disease, to maintain production and to ensure the welfare of the animals. The Toowoomba Veterinary Laboratory has actively encouraged the prudent use of antibiotics for animal treatment based on results of *in vitro* testing and field experience. Over the past three years, significant increases in antimicrobial resistance have generally not been observed. Exceptions to this are resistance to tetracycline and to a lesser extent, ampicillin.

References

- 1. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement, NCCLS M100-S12 Vol 21 No 1 NCCLS, Pennsylvania, USA; 2002.
- 2. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests; approved standard, 7th edition NCCLS M2-A7 Vol 20 No 1, NCCLS, Pennsylvania, USA; 2000.