Antimicrobial Resistance of Strains of Pasteurella hemolytica Isolated from Feedlot Cattle

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Pasteurella hemolytica is an important etiologic agent of bovine respiratory disease. Reggiardo reported that *Pasteurella spp.* comprised over 90% of the bacterial pathogens isolated from pneumonic lungs and of these isolates 85% of the strains were *P. hemolytica. P. multocida,* 15% of the strains, was usually recovered from chronic, rather than acute, pneumonic lungs.[1] Control of pasteurella pneumonia has been accomplished by the judicious use of antimicrobials approved for use in feedlot cattle. Hjerpe reported that *P. hemolytica* and *P. multocidia* were susceptible to many antimicrobials, usually resistant to streptomycin and erythromycin and always resistant to tylosin.[2]

Multiple resistance of strains of *P. hemolytica* has been reported. Small non-conjugal plasmids that code for resistance to tetracycline, dihydrostreptomyxin and possibly penicillin have been demonstrated in a strain of *P. hemolytica* isolated from feedlot cattle.[3]

During the past several years we have encountered an increasing frequency of respiratory disease in feedlot cattle that does not respond to conventional antimicrobial treatment. However, treatment with non-approved drugs, e.g. neomycin sulfate or chloramphenicol parenterally, resulted in excellent clinical response. This report deals with the resistance patterns of strains of *P. hemolytica* isolated from cattle with clinical signs of respiratory disease prior to treatment with antimicrobials in 1978, 1979, and 1980.

Materials and Methods

The cattle were purchased by an order buyer in Kentucky with no history of preconditioning, previous treatment or movement through sales channels. The cattle were processed on arrival at the Veterinary Medical Research Farm. Processing included an intranasal infectious bovine rhinotracheitis-parainfluenza 3 vaccine, an intramuscular attenuated bovine virus diarrhea vaccine, a seven-way clostridial product, implanting with zearolanone, 2.5 million units vitamin A, and deworming. No antibiotics were in the receiving ration nor were any antibiotics administered to the calves prior to the clinical diagnosis of respiratory disease and initiation of treatment. Nasal swabs were taken from the first calves with respiratory disease each of the three years. The swabs were taken at the time the calves were restrained for examination and clinical verification of the respiratory disease. The sample size varied from year to year; 29 in 1978, 22 in 1979 and 14 in 1980.

Results

A total of 65 samples (nasal swabs) were examined and the recovery rate of *P. hemolytica* was 78.5% or 51 strains (Table 1). More of the strains were resistant to streptomycin and tetracyclines than to any other antimicrobics tested, an incidence of resistance of 90%, 46 of 51 strains. Resistance to ampicillin was the next most prevalent, 21 of 51 or 41.2%. Twelve were resistant to sulfonamides, a 23.5% rate. Only a single isolate was resistant to chloramphenicol, and another to nitrofurans. Two strains were resistant to neomycin.

The distribution of patterns of resistance is presented in Table 2. Resistance to two or more antimocrobials was demonstrated for all of the resistant strains. The greatest frequency was resistance to both streptomycin and tetracyclines, 18 strains. The second most frequent pattern was 13 strains resistant to ampicillin and streptomycin. Eight strains were resistant to four and one to seven antimicrobials.

Minimum inhibitory concentrations (MIC) were determined for seven of the strains. (Table 3) The only result on resistance of the *P. hemolytica* strains to tylan is presented in the MIC data at the level of 25 ug/ml for all seven strains. The MIC was consistently at very high concentrations for oxytetracycline, sulfamethazine and streptomycin. The MIC to procaine penicillin, erythromycin and chloramphenicol were inconsistent.

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Summary of In Vitro Antibiotic Resistance of *Pasteurella hemolytica* Isolated from Pretreatment Nasal Swabs

Year	Number of Samples	Number of Isolates	Antibiotic Resistance						
			Ampicillin	Streptomycin	Tetracycline	Triple-Sulfa	Chloramphenicol	Nitrofuran	Neomycin
1978	29	23	13	19	19	2	—	—	—
1979	22	18	5	18	18	8	1	1	1
1980	14	10	3	9	9	2	—	—	—
Total	65	51	21	46	46	12	1	1	1

Discussion

The levels of resistance encountered in each of the three successive years markedly reduced the effectiveness of therapy with the commonly used antimicrobials: oxytetracycline, sulfonamides and penicillin. Animals that did not respond to treatment with one or more of these drugs would frequently respond to an antimicrobial not approved for use in feedlot cattle. The clinical observations parallel the laboratory data.

Distribution of Patterns of In Vitro Antibiotic Sensitivity of *Pasteurella hemolytica* Isolated from Pretreatment Nasal Swabs

Number of Isolates	Antibiotic Sensitivity						
	Ampicillin	Streptomycin	Tetracycline	Triple-Sulfa	Chloramphenicol	Nitrofuran	Neomycin
18	S	R	R	S	S	S	S
13	R	R	R	S	S	S	S
9	I	R	R	S	S	S	S
8	R	R	R	R	S	S	S
1	S	S	S	R	S	S	S
1	R	R	R	R	R	R	R
1	S	R	R	S	S	S	R

*R=resistant; S=susceptible; I=intermediate

A veterinarian is faced with several decisions when cattle with respiratory disease do not respond to the approved antimicrobials. Identification and treatment of sick cattle is most frequently the responsibility of the feedlot personnel in consultation with a veterinarian. The question must be answered as to the adequacy of the selection process to assure that cattle are being treated early enough in the disease process that a good response to treatment could be anticipated. If the cattle are depressed, weak, gaunt, have muzzles encrusted with a mucopurulent discharge and labored breathing the pneumonia is often so advanced that the response to treatment would be unsatisfactory regardless of the sensitivity of the pasteurella to antimicrobials. Further, many cattlemen do not appreciate the need to establish the proper dose of a given drug. Different formulations of the same drug may vary fourfold in the concentration, e.g. 50 mg per ml to 200 mg per ml. It is not unusual to find the same volume being given regardless of concentration. If neither identification of sick animals nor an accurate administration of treatment is a problem it is then necessary to decide when non-approved antibiotics should be used. Can lack of response ot treatment be considered an adequate basis for determining bacterial resistance or should supportive clinical bacteriologic findings be included?

The Food and Drug Administration, Bureau of Veterinary Medicine, has made it very clear that a veterinarian can administer or prescribe any antibiotic that he can obtain legally. Further, the Bureau of Veterinary Medicine indicates that an accurate diagnosis is pivitol in establishing the proper veterinarian-client relationship that is the underlying basis for using or dispensing non-approved drugs for the treatment of food producing animals. One must decide what constitutes an accurate diagnosis. Can lack of response to treatment with approved drugs be considered an adequate basis for determining that the casual bacteria are resistant? Are supportive clinical bacteriologic findings, including antibiograms, necessary in establishing an accurate etiologic diagnosis? It is a good policy to obtain nasal swabs from the first animals that are treated to determine the patterns of resistance of the bacterial flora of that specific population of cattle.

In Vitro Minimum Inhibitory Concentrations Studies on *Pasteurella hemolytica* Isolates Expressed as µg/ml

Isolate	Tylan	Procaine-Penicillin G	Oxytetracycline	Sulfamethazine	Erythromycin A	Chloramphenicol	Streptomycin
1	25	≤0.1	25	200	3.12	0.39	>200
2	25	>200	100	>200	3.12	1.56	>200
3	25	≤0.1	25	200	1.56	0.39	>200
4	25	≤0.1	25	>200	3.12	0.39	>200
5	25	25	25	>200	1.56	0.39	>200
6	25	50	25	>200	3.12	0.39	>200
7	25	12.5	25	>200	1.56	0.39	>200

Data courtesy of Pfizer Central Research—Groton, Conn.

The laboratory confirmation of resistance accomplishes two purposes. It establishes a firm diagnosis and is beneficial in selecting an antimicrobial to which the bacteria are sensitive. A veterinarian must also decide if he, in good

conscience, should prescribe a non-approved antibiotic. If effective drugs are not used, the potential loss to the client can be extremely costly due to death losses as well as loss of anticipated gains. If effective drugs are used some rational basis for dose, frequency and duration of administration and withdrawal time must be established. This information is not generally available in a documented form. The veterinarian must be sure that he has properly informed the client of the potential risks involved when documented data on residues and withdrawal times prior to slaughter are not available. This is particularly true with the aminoglycosides administered systemically that may result in detectable tissue residues for as long as four months, perhaps longer. The client must realize that the use of some drugs could alter marketing plans and that the additional time in the lot might be more costly than the losses that might be incurred because of lack of response to treatment.

The multiple resistance patterns to high concentrations of three or four antimicrobials is typical of plasmid mediated resistance. The MIC of the strains presented in Table 3 far exceed peak serum concentrations of tylan, oxytetracyclin, sulfamethazine and dihydrostreptomycin that would result from treatment at the recommended dose for feedlot cattle.[2] The reported peak serum concentrations for tylan following intramuscular (IM) administration of 2 mg/lb. is 0.6 ug/ml. after one hour and of 5 mg/lb. is 0.81 ug/ml. after two hours. The MIC of 25 ug/ml. indicates that tylan would have been totally ineffective. The MIC for oxytetracycline ranged from 25 to 100 ug/ml. as compared to serum concentrations 2.25 ug/ml. at two hours and 4.0 ug/ml. at four hours after IM administration of 2 mg/lb. and 5 mg/lb. respectively. Dihydrostreptomycin administered IM at 5 mg/lb. will give a serum concentration of 17 ug/ml. of serum within one-half hour as compared to the in vitro MIC greater than 200 ug/ml. Three of the strains would have been sensitive to penicillin with a MIC of 0.1 ug/ml. and an anticipated serum concentration at least 6 to 8 times greater with a dose of 2,000 IU/lb. IM. Similarly, three of the strains would have probably been sensitive in vivo to erythromycin and all but one to chloramphenicol.

Increasing the dosage level to achieve serum concentrations of a drug equivalent to the in vitro MIC might appear to be a solution. Griffin *et al*[4] reported on treating BRD in newly arrived feedlot calves with the recommended doses of 11 mg/kg at 33 mg/kg of oxytetracycline once daily. Alternate calves were treated with the two dosages. Forty-five calves were treated with the high dose and 51 with the low dose. Twenty calves in the high dose treatment group died and four in the low dose died. (P 0.1) The gross pneumonic lesions were similar in both treatment groups ranging from mild to sever fibrinous broncho-pneumonia. A positive, dose related, correlation was observed in the severity of microscopic lesions in the liver and kidney, and hepatorenal toxicity was considered the most probable cause of the significant difference in mortality between the two treatments. This report indicates that attempting to achieve high serum concentration of oxytetracycline is not a reasonable altermative to treating BRD caused by pasteurella. Seventeen of twenty strains of pasteurellas isolated in the above study were resistant to oxytetracycline in vitro.

The consistency of the resistance patterns during the three years raises critical questions. It was long thought that resistant strains of bacteria were at a disadvantage as compared to the wild or non-resistant strains. The mechanisms of resistance compete with other energy requirements of the bacterium for essential metabolic needs.

The successful bacterial pathogen must be able to reproduce, maintain virulence and combat the host's defense mechanisms. Beyond these basic characteristics pathogenic bacteria may also be able to resist the action of antimicrobials by diverting a portion of their metabolic processes to the production of resistance mechanisms. Tetracyclines must cross the cytoplasmic membrane to be active. Resistant bacteria produce additional protein compoments that block the transport of tetracyclines across the cytoplasmic membrane but make no contribution to other vital functions. The beta lactamase (penicillinase) produced by bacteria resistant to the beta lactam antibiotics serves no other function. The same is true of the alternative pathways to paramino benzoic acid metabolism that confers resistance to the sulfonamides. Each must be accomplished by expenditure of energy from a finite pool. The old dogma of bacterial selection indicated that removal of the selective pressure of antibiotics permitted the non-resistant strains to become the predominant flora. However, the shift to susceptible organisms may not occur. The resistant strains may, after years of selective pressure, be extremely well adapted to the host environment and the resistant flora maintained. Further, the resistance mechanisms may be maintained with the infrequent re-exposure to be antibiotic. Continuous selective pressure from the presence of antibiotics is not required. The ability of organisms to transfer genetic information within or between strains greatly enhances the retention of resistance genetic capabilities. Some of the genes that code for resistance may not function except when the antibiotic is present and thus do not put bacteria at any selective disadvantage when the antibiotic is removed.

The net result of these various mechanisms is the perpetuation of resistance in a given population. The data for the three year period suggests that resistant *P. hemolytica* has become the predominant flora capable of maintaining itself for long periods of time in the source population of cattle. The implications are obvious and on-going problems in the treatment of respiratory disease in feedlot cattle with the approved drugs can be anticipated.

References

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