Current Resistance Patterns of Bacterial Pneumonia Pathogens and Therapeutic Considerations*

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Summary

A standardized test of sensitivity (following DINx 58 940) to 10 chemotherapeutic agents was conducted on 49 *Pasteurella* and 10 *C. pyogenes* strains. These strains had been isolated from nasal or pulmonary secretions of calves with bronchopneumonia or from affected lung tissue of animals that had died. Resistance rates were determined on the basis of minimal inhibitory concentration or by the disk diffusion method. These were: for erythromycin 0%, trimethroprim/sulfadoxine 2.3%, penicillin G 20.5%, ampicillin 24.5%, kanamycin 24.5%, neomycin 32.7%, chloramphenicol 43.2%, sulfamethazine 54.5%, oxytetracycline 63.6%, and streptomycin 75.5%. *C. pyogenes* proved to be highly sensitive to penicillin G. Routine checking of resistance patterns is necessary for achieving effective tre tment of bronchopneumonia.

Introduction

The degree of resistance of the agents of bacterial diseases to chemotherapeutics is subject to regional and temporal variations. It is therefore necessary for the veterinarian to be informed of the current resistance pattern and to consider it in practice situations. Useful information can be obtained by sending samples to laboratory facilities or by using resistance tests in the individual practice. Relevant publications often do reflect regional conditions, but it may be indicated or necessary with certain herd problems to obtain more specific information about a particular resistance pattern.

In routine investigations the disk diffusion test (agar diffusion test) is utilized almost exclusively for the determination of resistance or sensitivity of bacterial disease agents. It is easy to use and also allows conclusions to be drawn about the definitive therapeutic test, i.e., the determination of minimum inhibitory concentration (MIC) of a certain medication for the pathogen. Assessment of the MIC is seldom carried out due to the relatively high cost. The minimum inhibitory concentration is defined as "the lowest concentration of a substance at which the growth of a bacterial population is inhibited under specified conditions" (Medical Standards Committee, 1979). If the minimum inhibitory concentrations determined for the pathogen are related to the achievable mean serum concentrations of the corresponding substances, that is the concentration in the middle of the administration intervals, then "sensitive", "moderately sensitive", and "resistant" groups can be established. The medical concept of resistance is easy to interpret. Pathogens with MIC values below the mean serum concentrations achievable with correspondingly high doses are considered sensitive, since the concentrations of the substance in the blood can be measured. Pathogens with MIC values over these blood levels are considered resistant. The blood levels are thus used as the standard, because the concentration of active substance at the desired site of activity is not always known or determinable. A division into "resistant", "moderately sensitive" or "sensitive" categories can also be made on the basis of the disk method, under strict standardization. Many experiments have shown that close correlations exist between inhibitory levels seen with this test and the MIC values.

Investigation

Methods

MIC determination and the disk diffusion test were used to establish the sensitivity or resistance of agents of bacterial pneumonia—*Pasteurella* and *C. pyogenes.* Material for the investigation consisted of nasal secretions taken with swabs, or pulmonary exudate obtained transtracheally from calves affected by bronchopneumonia, as well as samples from the diseased lungs of animals that died. A total of 49 strains of *Pasteurella* (31 x *P. multocida,* 18 x *P. hemolytica*) and 10 *C. pyogenes* strains were isolated.

The MIC determinations and the disk diffusion tests were conducted and evaluated according to the recommendations of the Medical Standards Committee (1979-1981). The test media used were: Mueller-Hinton agar (Difco) and Mueller-Hinton broth (Difco). This detail is important, in that values for the categories "sensitive", "moderately sensitive", and "resistant" and others depend on the growth medium used. Substances tested: penicilin G - potassium,

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1575 IU/mg (Bayer); oxytetracycline, 92.7% (Pfizer); chloramphenicol, 99.3% (Bayer); sulfamethazine, 99% (Impstoffwerk Friesoythe). Test disks used: ampicillin (10 ug, Oxoid), streptomycin (10 ug, Oxoid), neomycin (30 u, Oxoid), kanamycin (30 ug, Oxoid), erythromycin (15 ug, Oxoid).

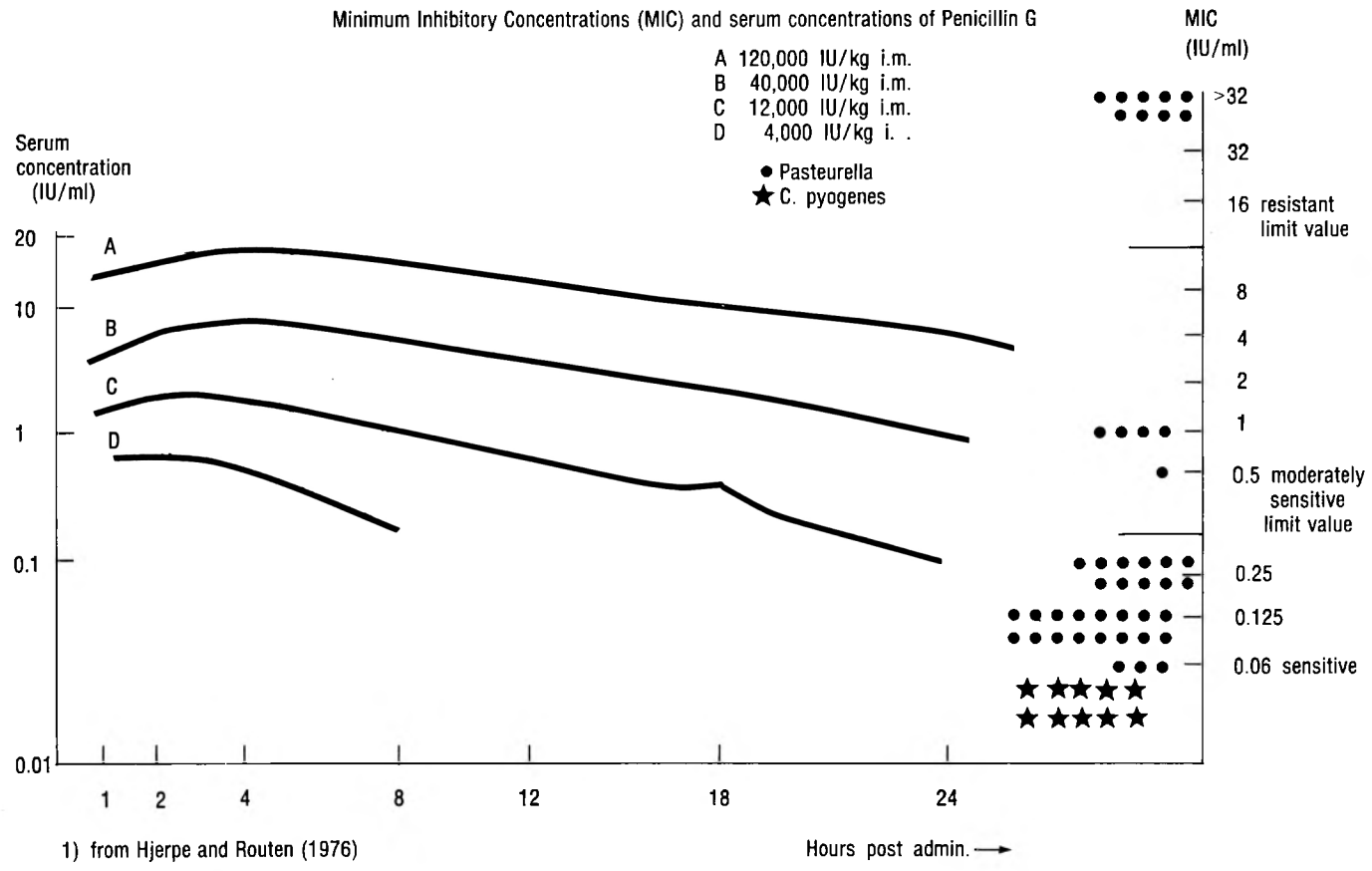
Results

The minimum inhibitory concentrations of penicillin G, oxytetracycline, and chloramphenicol for *Pasteurella* and *C. pyogenes* are graphically presented in figures 1-3, and are compared to the blood levels in calves as found in the literature (Hjerpe and Routen, 1976). The figures demonstrate what proportion of isolates show MIC values which are far above the blood levels achieved, and are thus considered resistant. Also, on the second ordinate axis, the limits of the "resistant" MIC region are labeled, which are the basis of the whole sensitivity test; these are derived from the blood levels achieved in human medical tests. Treatments of calves, carried out at dosage levels commonly used in practice did not fully achieve the corresponding levels of the MIC region designated "resistant". The resistance rates of *Pasteurella* for penicillin G, oxytetracycline and chloramphenicol amounted to 20.5%, 63.6% and 43.2% respectively (table 1). *C. pyogenes* proved to be highly sensitive to penicillin G.

The MIC values of sulfamethazine and trimethoprim/sulfadoxin for *Pasteurellae* (Fig. 4) showed a favorable resistance pattern for the trimethoprim-sulfonamide combination, in contrast to the sulfonamid alone (table 1). The limiting values used in determing resistance rates were based on levels reached in urine samples in human medicine.

The sensitivity testing of the isolates to ampicillin, streptomycin, neomycin, kanamycin and erythromycin was carried out using the disk diffusion test. Resistance rates of the *Pasteurellae* established with the disk diffusion test are listed in table 1, along with the values considered as the limits in determining resistance. The cut-off values given correspond to the standards set in DIN 58940 of 1981. Where no standard values were set in DIN 58940, the values established by the National Committee for Clinical Laboratory Standards—NCCLS—(1981) were used. The standardized sensitivity test for the bacterial pathogens, with these limit values, permits categorization, from a medical outlook, of the isolates as "sensitive" and "resistant". The resistance rates determined for *Pasteurellae* varied through a wide range of 0 (erythromycin) to 75% (streptomycin).

FIGURE 1: Serum concentrations of procaine penicillin G in relation to the minimum inhibitory concentrations determined in this experiment for **Pasteurella multocida** (29 strains), **Pasteurella hemolytica** (15 strains) and **Corynebacterium pyogenes** (10 strains).



1) from Hjerpe and Routen (1976)

FIGURE 2: Serum concentrations of oxytetracycline HCl in relation to the minimum inhibitory concentrations determined in this experiment for **Pasteurella multocida** (29 strains), **Pasteurella hemolytica** (15 strains), and **Corynebacterium pyogenes** (10 strains).

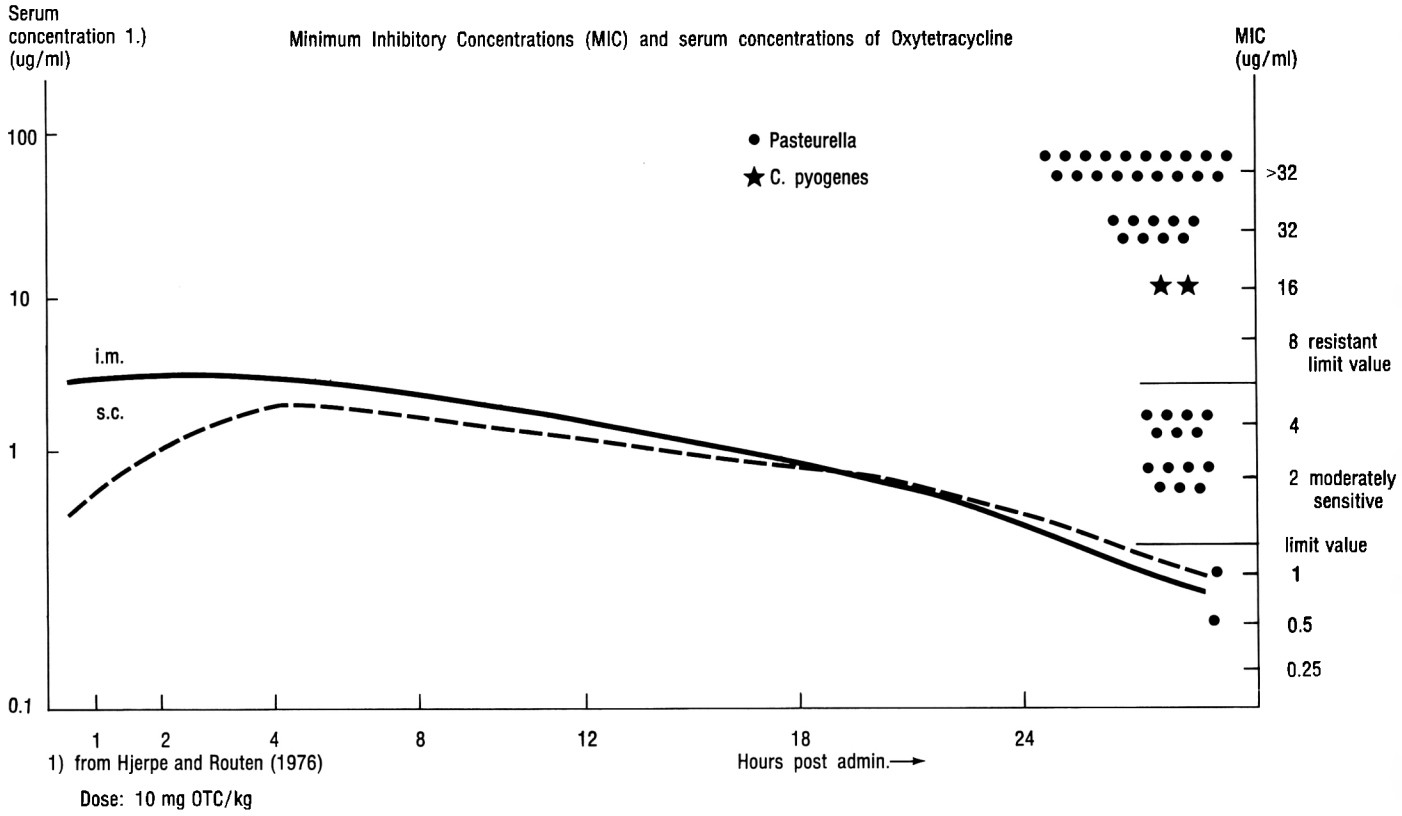


FIGURE 3: Serum concentrations of chloramphenicol in relation to the minimum inhibitory concentrations determined in this experiment for **Pasteurella multocida** (29 strains), **Pasteurella hemolytica** (15 strains), and **Corynebacterium pyogenes** (10 strains).

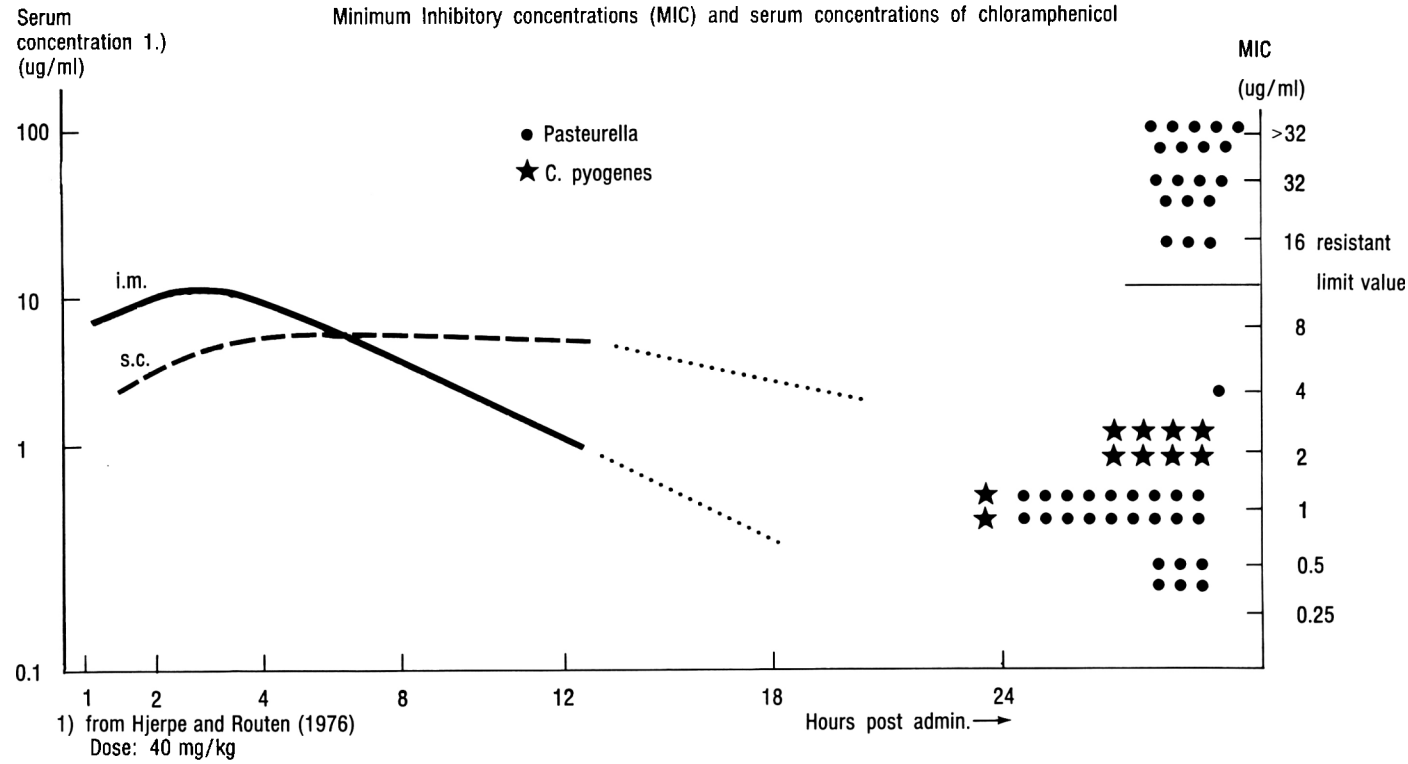
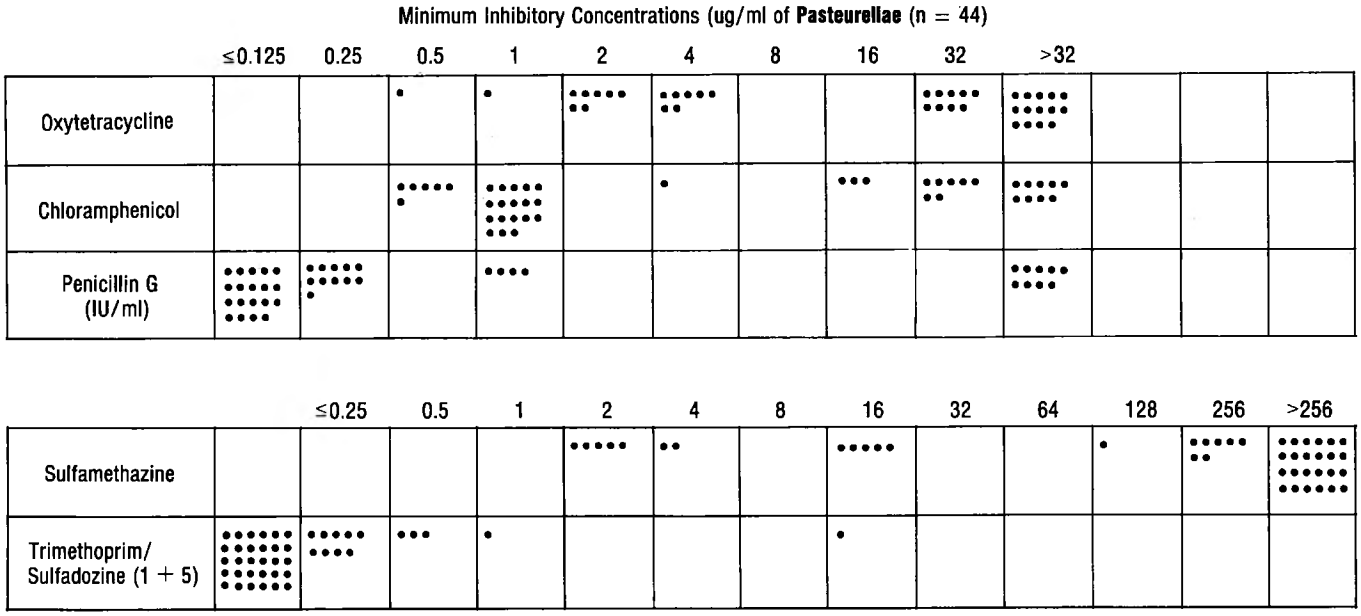


TABLE 1. Resistance of clinical isolates of **Pasteurella**

Chemotherapeutic agent	n	Percentage of resistance isolates: MIC Determination	Value Considered Resistant (ug/ml)	Disk Diffusion Test	Value Considered Resistant (mm)
Penicillin G	44	20,5	8*		
Oxytetracycline	44	63,6	4*		
Chloramphenicol	44	43,2	8*		
Sulfamethazin	44	54,5	256**		
Trimethoprim/Sulfadoxin	44	2,3	32***		
Ampicillin	49			24,5	14*
Streptomycin	49			75,5	11**
Neomycin	49			32,7	12**
Kanamycin	49			24,5	13**
Erythromycin	49			0	17*

*from DIN 58940, 1981
**from NCCLS, 1981
***from Dornbusch and Gezelius, 1980

FIGURE 4: Minimum inhibitory concentrations of the substances tested for **Pasteurella multocida** and **Pasteurella hemolytica.**



Discussion

Considerable progress has been made recently in efforts to standardize the methods for testing sensitivities of bacterial pathogens to chemotherapeutic agents, which has led to the adoption of German Standards (Medical Standards Committee, 1979 and 1981). These standards find application in human and veterinary medicine alike, although the assessment of values where pathogens are classified as resistant is based on blood levels achieved in human medicine. Figures 1-3 demonstrate that it cannot be simply assumed, that these levels are also reached in animals on treatment, without further concern. Reaching these levels required considerably higher doses than those formerly given. Formulations for an assessment scheme based on the situation found in animal medicine, do not exist. The resistance pattern of *Pasteurellae* can be characterized by classification of the 10 chemotherapeutics tested into 3 groups: erythromycin, trimethoprim-sulfadoxine, penicillin G, ampicillin and kanamycin, against which 0-24.5% of the strains were resistant, neomycin and chloramphenicol with resistance rates of 32.7% and 43.2%, and sulfamethazine, oxytetracycline and streptomycin, with especially high resistance rates of 54.4-75.5%. Comparison of the results obtained with this standardized sensitivity test and the results of other investigators is possible only to a limited

degree since the techniques and cut-off values used differ greatly, or are not clearly enough defined. Previous sensitivity tests of *Pasteurellae* show an extremely inconsistent picture. The resistance rates found with the disk diffusion test have varied for erythromycin between 8% and 72% (Larson, 1975; Hjerpe and Routen, 1976; Fales et al, 1982), for penicillin G between 0 and 61% (Fox et al, 1971; Chang and Carter, 1976) for tetracycline between 0 and 90% (Fox et al, 1971; Amstutz et al, 1982) and for chloramphenicol between 0 and 30% (Fox et al, 1971 ; and Hjerpe and Routen, 1976). For other substances, resistance rates with similar variability have been reported. How much these differences are due to regional variations and how much originates from discrepancies in the methods and assessment criteria used cannot be determined. This underscores the importance of a standardized sensitivity test.

The results of the MIC determination presented here correspond with clinical experience. In the region of origin of these isolates trimethoprim-sulfonamide combinations, penicillin G in doses of 20,000-40,000 IU/kg BW, ampicillin, kanamycin and erythromycin seem very suitable at present, while with chloramphenicol, and even more so with oxytetracycline, sulfamethazine, and streptomycin, a high percentage of resistant organisms can be expected. Sensitivity testing should help guard against the use of medications towards which the offending bacterial agent is sensitive only at levels which "as a rule are not achievable under treatment conditions using systemic therapy, so that antibacterial effect cannot be expected in vivo" (Medical Standards Committee, 1981). From the group of substances which seem appropriate for therapy, the choice is based on medical considerations, especially those concerning pharmacokinetics. The concentration of the compound in blood may be higher or lower than that in different tissues, in particular at the target tissue.

The localization and regional nature of our materials somewhat limit the predictive value of this experiment. However, the results do show that in practice, at least regionally, considerable resistance to different chemotherapeutics must be reckoned with. Ongoing monitoring of the resistance pattern is necessary for effective treatment of bronchopneumonia.

English translation by Dr. Franklin Garry, Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.