Live Animal Sampling for Antibacterial Susceptibility Testing

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Abstract

The value of *in vitro* antibacterial susceptibility testing depends on the relevancy of the microbiological samples collected. Those from the lower respiratory tract are most likely to contain etiologic pathogens but their collection requires a high level of expertise and specialized equipment. Therefore, collection of tracheal swabs is recommended. Samples should be refrigerated and desiccation should be avoided. Selection of antibacterial agents for initial therapy of BRD should be based on microbiological testing of samples collected from live animals prior to therapy. Selection of antibacterial agents to be used when initial treatment fails can be based on results of susceptibility testing conducted on treatment failures.

Correct interpretation of agar disk diffusion or MIC data depends not only on an understanding of the correlation between *in vitro* susceptibility and *in vivo* efficacy, but also the origin of the samples from which offending bacteria are cultured. Ideally, samples collected for bacteriological culture and isolation should: (1) contain the etiologic pathogen and not be contaminated with inconsequential isolates; (2) be collected, preserved, packaged, and transported in such a manner that the viability of microorganisms is maintained; and (3) yield isolates that are representative etiologic agents of the disease condition. These requirements are sometimes difficult to satisfy in the managerial and physical environment in which feedlot cattle are maintained.

Identification of etiologic pathogens

Microorganisms that have been implicated as etiologic agents of bovine respiratory disease (BRD) of feedlot cattle include; *Pasteurella haemolytica, Pasteurella multocida, Mycoplasma spp, Pseudomonas aeruginosa, Haemophilus somnus,* and *Actinomyces pyogenes. P. haemolytica* is most frequently isolated from the lungs of feedlot cattle with bacterial pneumonia and is considered to be the most common and important causative agent of BRD.[1] However, other organisms, such as *Mycoplasma* spp and *Actinomyces pyogenes* are increasingly being isolated from cattle that have died from BRD. Whether they are primary etiologic agents or secondary pathogens, their isolation, identification and *in vitro* susceptibility testing are essential for selection of an appropriate therapeutic strategy. Indeed, an assumption that *P. haemolytica* is the only important causative organism in a specific feedlot may lead to inappropriate drug selection and high treatment failure rates.

Selection of sampling site

P. haemolytica normally inhabits the nasal mucosa of healthy cattle, but when host defense mechanisms are compromised by physical stress or viral infection the organism multiplies rapidly and descends into the lung causing fibrinopurulent pneumonia.[2,3] Therefore, collection of samples from the nasal mucosa should yield isolates that are representative of those colonizing the lung. However, there is evidence that the microbiology of the nasal cavity is quite different from that of pneumonic lungs. Although *P. haemolytica* is a normal inhabitant of bovine nasal mucosa, the serotypes colonizing this site are not necessarily those responsible for pneumonia. In comparison with diseased animals, healthy animals have a higher ratio of serotype 2 to serotype 1 isolations.[4] Serotype 1 biotype A is usually implicated in pneumonic pasteurellosis. Thus, determination of *in vitro* antibacterial susceptibilities of isolates cultured from the nasal cavity of healthy cattle is unlikely to provide useful information. This is unfortunate because it precludes screening of cattle when they are initially processed on arrival at the feedlot. Even when animals are stressed and *P. haemolytica* serotype 1 has overgrown other serotypes and bacteria in the nasopharyngeal region, nasal swab samples may not adequately represent bacteria colonizing the lung. In several experiments, antimicrobial susceptibilities of *P. haemolytica* isolated from the upper respiratory tract have been found to be different from those of the lower respiratory tract. For example, in one study nasopharyngeal isolates had a higher incidence of resistance to penicillin G, ampicillin, and oxytetracycline than those isolated from pneumonic lungs.[5] Unfortunately, the value of isolating pathogens from the lower respiratory tract is compromised by the inaccessibility of this region in live animals and the difficulty with which such samples are collected. However, samples from the laryngotracheal region showed also provide information that is relevant to the lung.

Methods of sample collection and storage

Great care should be exercised to ensure that samples contain relevant pathogens and are not contaminated with inconsequential microorganisms. Swabs must not come into contact with any surfaces other than relevant nasopharyngeal or respiratory mucosa and lavage buffers must be directly delivered to and collected from the region of interest. Nasal swabs must be inserted through the nostril for collection of samples from the nasal cavity; samples of nasal discharge from the exterior of the muzzle are likely to be contaminated with other microorganisms that overgrow the offending pathogen when cultured. These contaminants may suppress the growth of the etiologic bacterium or may so confuse diagnosis that additional time-consuming isolations become necessary. Contaminants present a particular problem when the offending pathogen grows fastidiously on diagnostic media. Laryngotracheal samples are best collected using equine uterine swabs. These samples can be collected by restraining the animal using a head clamp and nose tongs, depressing the dorsum of the tongue, and illuminating the laryngeal folds. Lung lavage is accomplished in similar fashion: a flexible plastic tube approximately 4 foot in length can be passed through the outer sleeve of an equine uterine catheter once it has been positioned in the trachea and the swab removed. When the flexible catheter cannot be threaded any further into the distal airways, 50 ml of sterile buffer is injected and then immediately aspirated. Lung lavage samples are collected from the most relevant tissue region involved in bovine respiratory disease and, when collected aseptically, are least likely to contain inconsequential contaminants. Although lung lavage is easily accomplished with trained personnel and suitable cattle handling facilities, it is not a practical option in the feedlot environment because it is time consuming and subject to many methodological errors. Collection of nasal samples is most easily achieved but the results are the least reliable because of the accessibility of this region to contaminants. Therefore, tracheal swabs are recommended because they provide a balance between ease of collection and relevancy of results.

The two most important considerations in shipping samples for microbiological isolation are: (1) prevention of desiccation; and (2) refrigeration. Desiccation is most likely to occur when nasal or laryngotracheal swabs are stored or transported without media. Culturette-type swabs that contain pre-packaged media provide a convenient means of avoiding desiccation and contamination. Refrigeration is best achieved by packing samples in styrofoam-insulated leak proof containers with gelform ice packs. Appropriately packaged samples must be analyzed as soon as possible after collection and, therefore, should be hand-delivered to the diagnostic laboratory or express mailed.

Effect of antibacterial therapy on susceptibility

Ideally, samples for antibacterial sensitivity testing should be collected from pneumonic animals prior to treatment. In particular, selection of antibacterial agents based solely on culture and susceptibility testing of samples collected from dead animals should be avoided. The antibacterial susceptibilities of pathogens isolated from these treatment failures are unlikely to be representative of pathogens infecting most cattle in the feedlot. Similarly, samples collected from live animals that have not responded satisfactorily to antibacterial therapy may provide useful information concerning the identities and antibacterial susceptibilities of pathogens infecting the particular animal, but are not representative of the pathogenic bacterial population as a whole. Obviously, each animal suffering from BRD cannot be subjected to culture and antibacterial susceptibility testing before initiation of antibacterial therapy. Therefore, it is recommended that periodic live sampling of animals diagnosed with BRD and not yet treated with antibacterial agents be used to select antibacterial agents to be used as initial therapy and that data derived from subsequent testing of antibacterial-treated animals be used to identify suitable agents for treating those animals that do not respond to initial therapy. The information used to formulate antibacterial selection priorities should be updated at least twice a year, in the spring and the fall, and should only be applied to the reference population. Depending of the geographic origin of cattle shipped to a feedlot, specific pens or groups of pens may have to be tested to provide relevant data. However, considering the diverse origin of feedlot cattle and the probability that many cattle are exposed to etiologic pathogens before they even enter the feedlot, it may not be possible to monitor antibacterial susceptibilities of a relevant population of pathogens in a timely manner. Hopefully, antibacterial agents with sufficiently broad spectra of activity against most isolates of etiologic bacteria, especially *P. haemolytica*, from a range of geographical origins can be identified. Such an approach to selection of antibacterial therapy is more likely to result in satisfactory treatment responses than basing decisions on purely empirical evidence or simply using the product that was most recently approved.

References

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