This study was initiated after an apparent rise in the number of isolates of Gallibacterium anatis in the poultry industry was observed over the last 5 yr. Once classified as a Pasteurella, this organism has not typically been perceived as a major pathogen in commercial poultry, at least not in the meat-type poultry industry in the southern United States. Gallibacterium anatis is known to colonize the upper respiratory tract and lower reproductive tract of normal chickens, but it has also been experimentally shown to induce natural infection in the chicken model (Bojesen et al., 2004).

Gallibacterium anatis has had many names over the course of history such as Pasteurella hemolytica, Pasteurella anatis, Mannheimia haemolytica, and Actinobacillus salpingitidis, but was recently established as a new genus, Gallibacterium, within the family Pasteurellaceae (Christensen et al., 2003). This complicates the interpretation of the past literature, as there are reports that apparently describe this organism in poultry, but use the earlier classifications (Lin et al., 2001; Malik et al., 2005).

Gallibacterium anatis became of interest when it was found to occur in pure culture from “problem” broiler and breeder flocks. It was isolated from joints, wattles, lungs, abdomens, hearts, and other viscera. In breeder flocks, G. anatis appears to present very similarly to what typically is seen with Pasteurella multocida (fowl cholera) systemic infections (nagging mortality, decreased egg production, peritonitis, and airsacculitis). Antimicrobial therapy commonly administered to these fowl cholera-like flocks (e.g., penicillin or tetracyclines) proved to be ineffective. The antibiograms shown in this study demonstrate why this occurred. Bojesen et al. (2004) reported that G. anatis has been recovered in pure culture from a range of pathological lesions in chickens, including septicemia, oophoritis, follicle degeneration, salpingitis, peritonitis, and respiratory tract infections. Neubauer et al. (2009) showed that G. anatis isolates are prevalent in layers with reproductive...
disorders and that they play a role in the disorders. According to these authors, isolates of *G. anatis* from different locations in the body were highly similar; indicating that isolates residing in their natural habitat (upper respiratory system) may cause respiratory disorders or systemic disease under certain conditions. In a recent publication, it was shown that nasal inoculation with *G. anatis* caused microscopic lesions in the trachea, lungs, air sacs, and liver (Zepeda et al., 2010). They concluded that *G. anatis* may be considered a primary pathogen for the respiratory tract of chickens and that liver damage detected may suggest the possibility of blood dissemination not with standing the nasal route of inoculation. This report compares the number of isolations and the antibiograms of *G. anatis* and *P. multocida* over the past 5 yr from chickens in Mississippi.

**Materials and Methods**

Samples were routinely submitted to the Poultry Research and Diagnostic Laboratory for microbiological culture and sensitivities. Samples (approximately 300 tissues or swabs) were initially inoculated onto 5% sheep blood plates, Columbia CNA with 5% sheep blood plates, and MacConkey agar plates. Plates were then incubated for 18 to 24 h at 37°C.

**Sensititer System Identification Protocol**

Suspect bacterial colonies were selected and identified by analysis with the Sensititer ARIS 2X system (Trek Diagnostic Systems Inc., Cleveland, OH) using the Sensititer GNID panel. The Sensititer system identifies *G. anatis* as *M. haemolytica* and this is what was reported by this laboratory for several years. *Mannheimia haemolytica* is not normally associated with poultry disease, so to confirm the identification, a section of the 16S rDNA gene was sequenced. Thirty isolates picked from the years 2006 to 2011 were selected for confirmation by sequencing. Single bacterial colonies were picked and inoculated into 3 mL of brain heart infusion and grown overnight at 37°C. The DNA was extracted from 1 mL of the broth cultures using the DNasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer’s protocol. Degenerate primers Florida (corresponding to positions 8–27 and 575–556; Kotilainen et al., 1998; Wilbrink et al., 1998) of the *Escherichia coli* 16S rDNA gene) were used to amplify a section of the 16S rDNA gene by PCR. The amplicons were sequenced by a commercial firm (Eurofins MWG, Huntsville, AL). The 16S rDNA sequences were compared with those in the GenBank database by using the Basic Local Alignment Search Tool (BLAST) search tool (Altschul et al., 1997). The BLAST search identified the poultry isolates as *Gallibacterium anatis*. Selected bovine isolates identified as *M. haemolytica* were also checked by DNA sequencing, and all were confirmed as *M. haemolytica*.

**Sensititer System Antibiotic Sensitivity Protocol**

Sensitivities were determined for the following antibiotics: β-lactams (amoxicillin, ceftiofur, and penicillin), lincomamide (clindamycin), tetracyclines (doxycycline, minocycline, oxytetracycline, and tetracycline), fluoroquinolones (enrofloxacin), macrolides (erythromycin and tylosin tartrate), florfenicol, aminoglycosides (gentamicin, neomycin, and streptomycin), novobiocin, spectinomycin, and sulfonamides (sulfamethoxazole/trimethoprim, sulphanilamide, and sulphathiazole).

Antibiotic minimum inhibitory concentration (MIC) sensitivities were determined utilizing a broth microdilution technique with the Sensititer AVIAN1F panel (Trek Diagnostic Systems, Cleveland, OH) following the suggested manufacturer protocols. The Sensititer AVIAN1F was used because it is the standard plate used in our diagnostic laboratory to characterize the antibiograms of poultry bacterial pathogens. The panel and concentration ranges included amoxicillin (0.25 to 16 mg/mL), ceftiofur (0.25 to 4 mg/mL), clindamycin (0.5 to 4 mg/mL), doxycycline (0.25 to 8 mg/mL), enrofloxacin (0.12 to 2 mg/mL), erythromycin (0.12 to 4 mg/mL), florfenicol (1 to 8 mg/mL), gentamicin (0.5 to 8 mg/mL), minocycline (0.25 to 8 mg/mL), neomycin (2 to 32 mg/mL), novobiocin (0.5 to 4 mg/mL), oxytetracycline (0.25 to 8 mg/mL), penicillin (0.06 to 8 mg/mL), spectinomycin (8 to 64 mg/mL), streptomycin (8 to 1024 mg/mL), sulfamethoxazole/trimethoprim (2 to 38/0.5 to 9.5 mg/mL), sulfadimethoxine (32 to 256 mg/mL), tetracycline (0.25 to 8 mg/mL), and tylosin (2.5 to 20 mg/mL). Sensititer plates were read by the Sensititer Automated Reading and Incubation System (Trek Diagnostic Systems Inc.). All results were generated using the Sensititer software (Trek Diagnostic Systems Inc.).

**Interpretation of Results**

Interpretation of the MIC values was based on the Sensititer database supplied by TREK Diagnostic Systems, which is based on the Clinical and Laboratory Standards Institute (2002) guidelines for bacteria isolated from animals. The MIC breakpoints for resistance/susceptibility were amoxicillin (≥16/≤3 mg/mL), ceftiofur (≥8/≤2 mg/mL), clindamycin (≥4/≤0.5 mg/mL), doxycycline (≥16/≤4 mg/mL), enrofloxacin (≥2/≤0.25 mg/mL), erythromycin (≥8/≤0.5 mg/mL), florfenicol (≥8/≤2 mg/mL), gentamicin (≥16/≤4 mg/mL), minocycline (≥8/≤1 mg/mL), neomycin (≥32/≤0 mg/mL), novobiocin (≥4/≤1 mg/mL), oxytetracycline (≥8/≤1 mg/mL), penicillin (≥0.25/≤0.125 mg/mL), spectinomycin (≥64/≤16 mg/mL), streptomycin (≥64/≤32 mg/mL), sulfamethoxazole/trimethoprim [sulfamethoxazole (≥76/≤38 mg/mL)/trimethoprim (≥4/≤2 mg/mL)], tetracycline (≥8/≤1 mg/mL), and tylosin tartrate (≥20/≤5 mg/mL).
RESULTS AND DISCUSSION

A total of 291 isolates of *G. anatis* (84) and *P. multocida* (207) were identified from November 2006 to December 2011 (Figure 1). The number of isolations per year of *G. anatis* gradually increased from \( n = 6 \) in 2006 to 2007 to \( n = 28 \) in 2010 to 2011. These isolates came from both broiler flocks (44 isolates) and broiler breeders (40 isolates) in near equal numbers. Both bird types show a similar increasing trend in the number of *G. anatis* isolations. For *P. multocida*, the number of isolations per year increased from \( n = 33 \) in 2006 to 2007 to \( n = 56 \) in 2009 to 2010. The number of isolations then decreased in 2010 to 2011 to \( n = 32 \). All *P. multocida* isolations were from broiler breeder type chickens.

**G. anatis**

For the isolates of *G. anatis* identified in this study, the antibiotic(s) with the highest percentage of susceptibility were gentamicin (93% S; \( S = \) sensitive; \( I = \) intermediate; \( R = \) resistant), enrofloxacin (93% S), ceftiofur (90% S), florfenicol (86% S), sulfamethoxazole/trimethoprim (83% S), and sulfathiazole (82% S).

*Gallibacterium anatis* was resistant to novobiocin (100% R), tylosin (100% R), clindamycin (97% R), tetracycline antimicrobials (range of 80–90% R), and penicillin (70% R). There was intermediate susceptibility to spectinomycin (89% I). Isolates of *G. anatis* showed mixed sensitivity for amoxicillin (43% S, 21% I, 36% R), erythromycin (57% I, 43% R), neomycin (64% S, 22% I, 14% R), streptomycin (75% S, 4% I, 21% R), and sulphadimethoxime (43% S, 14% I, 43% R). Sensitivities for *G. anatis* are shown in Figure 2.

*Gallibacterium anatis* isolates were sensitive to ceftiofur (90% S), enrofloxacin (93% S), gentamicin (93% S), florfenicol (86% S), sulfamethoxazole/trimethoprim (83% S), and sulfathiazole (82% S). These results are similar to those of Malik et al. (2005) who reported *M. haemolytica* chicken isolates with sensitivities to ceftiofur (100% S), enrofloxacin (96–100% S), florfenicol (92–100% S), and sulfamethoxazole/trimethoprim (93–100% S). Similarly, Lin et al. (2001) also reported strong sensitivity in *G. anatis* isolates from chickens to ceftiofur and enrofloxacin. Similar findings have also been reported in other species. Berge et al. (2006) reported 100% sensitivity to ceftiofur and florfenicol from *Mannheimia haemolytica* isolates taken from sheep and goats. High sensitivities to ceftiofur, enrofloxacin, gentamicin, and sulfamethoxazole/trimethoprim have been reported for sheep isolates of *Pasteurella hemolytica* (Diker et al., 1994). *Mannheimia haemolytica* isolated from swine were reported to have 100% sensitivity to florfenicol (Priebe and Schwarz, 2003). Sensitivities in *M. haemolytica* isolates from this study to ceftiofur, florfenicol, gentamicin, and enrofloxacin are similar to findings previously reported in cattle (Post et al., 1991; Mevius and Hartman, 2000; Aslan et al., 2002; Hendriksen et al., 2008; Katsuda et al., 2009; Watts and Sweeney, 2010). However, contrasting reports exist for *M. haemolytica* isolates from cattle that demonstrate variable to low sensitivities to sulfamethoxazole/trimethoprim (Mevius and Hartman, 2000; Hendriksen et al., 2008).

There was intermediate sensitivity to spectinomycin in which over 89% of the isolates were moderately sensitive. Malik et al. (2005) reported that only 0 to 50% of chicken isolates were sensitive to spectinomycin. In cattle, Schwarz et al. (2004) reported that 98% of the isolates were sensitive, and Watts et al. (1994) and Watts and Sweeney (2010) reported that 83% of the isolates were sensitive.

*Gallibacterium anatis* isolates from this study revealed resistance to novobiocin (100% R), tylosin (100% R), clindamycin (97% R), tetracycline antimicrobials (range of 80–90% R), and penicillin (70% R). Similar findings in chickens have been reported for penicillin (60–100% R) and tetracycline (72–100% R) by Malik et

![Figure 1. Total isolations of *Gallibacterium anatis* and *Pasteurella multocida* from 2006 to 2011.](image-url)
al. (2005). In contrast to our findings, Lin et al. (2001) reported moderate sensitivity to tetracycline. They did find strong resistance in chicken isolates to penicillin, however. Isolates from cattle have been reported to be resistant to penicillin, tylosin, and tetracyclines (Post et al., 1991; Watts et al., 1994; Mevius and Hartman, 2000; Hendriksen et al., 2008; Johnson et al., 2011). In contrast, Berge et al. (2006) reported only a 5% resistance to tetracyclines in sheep and goat isolates.

Isolates of *G. anatis* showed mixed sensitivity results for amoxicillin (43% S, 21% I, 36% R), erythromycin (57% I, 43% R), neomycin (64% S, 22% I, 14% R), streptomycin (75% S, 4% I, 21% R), and sulphadimethoxime (43% S, 14% I, 43% R). Lin et al. (2001) also reported strong sensitivity to amoxicillin in chickens. Berge et al. (2006) found that 100% of their sheep and goat isolates were sensitive to amoxicillin. Hendriksen et al. (2008) found that most of their cattle isolates were also sensitive to amoxicillin. In contrast, Mevius and Hartman (2000) found their cattle isolates to be resistant to amoxicillin. For erythromycin, Malik et al. (2005) reported that none of their chicken isolates were sensitive. This was similar to the findings of Lin et al. (2001), who found strong resistance to erythromycin. Watts et al. (1994) also reported a very low sensitivity of cattle isolates to erythromycin. In contrast to our findings, Lin et al. (2001) reported only slight sensitivity to streptomycin and neomycin. Also in contrast to our findings, Post et al. (1991) reported that cattle isolates showed moderate sensitivity to streptomycin and streptomycin. Results similar to ours were reported regarding sulphadimethoxime sensitivity in chicken isolates (Mevius and Hartman, 2000) and in cattle isolates (Post et al., 1991).

**P. multocida**

For the isolates of *P. multocida* identified in this study, the antibiotic(s) with the highest percentage of susceptibility were doxycycline (99% S), minocycline (99% S), sulfamethoxazole/trimethoprim (96% S), ceftiofur (95% S), florfenicol (94% S), amoxicillin and enrofloxacin (93% S), sulfathiazole (92% S), neomycin (89% S), oxytetracycline and tetracycline (86% S), novobiocin and sulphadimethoxine (85% S), and gentamicin (79% S). Pasteurella multocida isolates were resistant (97% R) only to clindamycin and tylosin and had intermediate sensitivity to erythromycin (78% I) and spectinomycin (87% I). Isolates of *P. multocida* showed mixed sensitivity for penicillin (54% S, 30% I, 16% R) and streptomycin (22% S, 18% I, 60% R). Sensitivities for *P. multocida* are shown in Figure 3.

In sharp contrast to *G. anatis* isolates, *P. multocida* isolates showed moderate to high sensitivity to β-lactam, novobiocin, and tetracycline antimicrobials, but had similar antibiograms for the other antimicrobials. Our chicken isolates were sensitive to doxycycline (99% S), minocycline (99% S), sulfamethoxazole/trimethoprim (96% S), ceftiofur (95% S), florfenicol (94% S), amoxicillin and enrofloxacin (93% S), sulfathiazole (92% S), neomycin (89% S), oxytetracycline and tetracycline (86% S), novobiocin and sulphadimethoxime (85% S), and gentamicin (79% S). Similar findings have been widely reported in chickens, and recently by Huang et al. (2009) for gentamicin, amoxicillin, ceftiofur, enrofloxacin, florfenicol, spectinomycin, tetracycline, and sulfamethoxazole/trimethoprim.

There are also similar findings in other species. Berge et al. (2006) reported a high sensitivity to amoxicillin (100% S), ceftiofur (100%), florfenicol (100% S), and tetracyclines (95%) in sheep and goats. High sensitivity to ceftiofur in cattle has been previously reported, as well (Watts et al., 1994; Mevius and Hartman, 2000; Pribe and Schwartz, 2003; Hendriksen et al., 2008; Watts and Sweeny, 2010). In addition, there are reports in cattle (Mevius and Hartman, 2000; Hendriksen et al., 2008; Watts and Sweeny, 2010) and in swine (Pribe and Schwarz, 2003) of high sensitivity to florfenicol. There have been mixed reports regarding sensitivity to gentamicin in cattle. Mevius and Hartman (2000) re-

**Figure 2.** Antibiotic sensitivities of *Gallibacterium anatis* isolates.
reported a high sensitivity (84–98% S), whereas others (Post et al., 1991) reported only a moderate sensitivity. Mevius and Hartman (2000) also reported a high sensitivity (84–98% S) to neomycin, which is similar to our findings. There are also mixed reports for sensitivity to amoxicillin. In sheep and goats by Berge et al. (2006) and in cattle by Hendriksen et al. (2008), there are reports of high sensitivity to amoxicillin, whereas others (Mevius and Hartman, 2000) reported variable results (47–84% S) in cattle. In contrast, Hendriksen et al. (2008) reported a variable sensitivity to enrofloxacin in cattle, but others (Mevius and Hartman, 2000) reported high sensitivity (84–98% S). Sensitivity to tetracycline similar to ours was reported in sheep and goats (Berge et al., 2006). Variable to high resistance to tetracycline has been reported in cattle (Post et al., 1991; Watts et al., 1994; Mevius and Hartman, 2000). Similar findings in cattle for sulfamethoxazole/trimethoprim were reported by Hendriksen et al. (2008), but variable sensitivities were reported by (Mevius and Hartman, 2000). A conflicting report (Post et al., 1991) reported high resistance to sulfadimethoxine in cattle in contrast to the high sensitivity observed in our chicken isolates.

Similar to reports in cattle (Post et al., 1991; Watts et al., 1994), there was intermediate sensitivity to erythromycin (78% I) and spectinomycin (87% I). There are also conflicting reports for spectinomycin reported in chickens (Huang et al., 2009) and in cattle (Mevius and Hartman, 2000; Schwarz et al., 2004), which showed high sensitivities (98% S, 84–98% S, 94% S, respectively). There was high resistance (97% R) only to clindamycin and tylosin. The tylosin findings are similar to those reported in cattle by Post et al. (1991). Isolates showed mixed sensitivity results for penicillin (54% S, 30% I, 16% R) and streptomycin (22% S, 18% I, 60% R). Similar to our findings, Post et al. (1991) reported only a moderate sensitivity to penicillin.

**Conclusion**

The overall rate of isolation of *G. anatis* gradually increased from 2006 to 2011. These isolates came from about as many broiler flocks as broiler breeders. For *P. multocida*, the rate of isolation increased from 2006 to 2010, but decreased through 2011. *Pasteurella multocida* isolates all came from broiler breeder type chickens. The decrease in isolations in 2011 for *P. multocida* may be due to producers recognizing the clinical signs for this agent and not submitting chickens/samples for all incidences of the disease. However, the clinical signs for *G. anatis* infections are very similar and it would be expected that the rate of isolation for this bacteria would also have decreased. Because the rate of *G. anatis* isolations increased during this period, the logical conclusion is that the incidence of *G. anatis* increased from 2006 to 2011 relative to the incidence of *P. multocida*.

Here we are reporting that the incidence of *G. anatis* has increased over the 5-yr period of 2006 to 2011 relative to the incidence of *P. multocida* as viewed by the number of isolations from samples submitted to the PRDL. It is also of note that *G. anatis* was isolated from both broiler flocks and broiler breeders and that *P. multocida* was only isolated from broiler breeder type chickens. One area of future research will be in the characterization of the virulence mechanisms of the 2 pathogens that could explain why there were no isolations of *P. multocida* from the broiler flocks during this period.

The 2 bacterial pathogens presented different antibiograms. *Gallibacterium anatis* demonstrated almost complete resistance to novobiocin, tylosin, lincomamide, and tetracycline antimicrobials, and moderate to high sensitivity to sulfonamides, fluoroquinolones, and florfenicol. They presented intermediate sensitivity for spectinomycin and erythromycin, and variable resistance to β-lactam and aminoglycoside antimicrobials.
In sharp contrast, *P. multocida* demonstrated moderate to high sensitivity to β-lactam, novobiocin, and tetracycline antimicrobials, but had antibiograms similar to *G. anatis* for the other antimicrobials. Although it is true that there are most definitely constitutive reasons for resistance, one has to consider that the 2 organisms of focus in this manuscript are both from the family *Pasteurellaceae* and are very similar. One would expect that they would have very similar antibiograms.

The fact that they do not makes for the basis and the original interest of the paper. In this study, we chose not to focus on resistance changes over time nor acquired vs. constitutive resistance, but rather highlight 2 similar organisms causing very similar disease with 2 very different antimicrobial treatment options. Because treatment without a culture and sensitivity commonly occurs in the industry, we are attempting to warn producers/veterinarians of this potential scenario when dealing with fowl cholera-type flocks.

This study demonstrates that there is a need for continued monitoring of the antibiograms of poultry bacterial pathogens to periodically re-evaluate the effectiveness and use of antibiotics in the industry. By monitoring the flocks and identifying the pathogen and characterizing its antibiogram using culture and sensitivity assays, effective antimicrobials can be used by producers to treat the bacterial agent. If only appropriate antibiotics are used in our poultry flocks, we can help to ensure we do not abuse their use, thus helping to maintain antibiotic availability in our very limited arsenal of therapeutics and reducing the cost to producers when inappropriate antibiotics are used.

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**REFERENCES**


