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Susceptibility of *Arcanobacterium pyogenes* from different sources to tetracycline, macrolide and lincosamide antimicrobial agents

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Abstract

Chlortetracycline, oxytetracycline, and the macrolide, tylosin, are extensively used for growth promotion and disease prophylaxis in the cattle and swine industries in the US. *Arcanobacterium pyogenes*, a common inhabitant of the mucosal surfaces of cattle and swine, is also a pathogen associated with a variety of infections in these animals. A broth microdilution technique was used to determine the antimicrobial susceptibility of 48 *A. pyogenes* isolates to macrolides, lincosamides and tetracyclines. The MIC₅₀ and MIC₉₀ for chlortetracycline were 0.12 and 8 mg/l, respectively. Similarly, the MIC₅₀ and MIC₉₀ for oxytetracycline were 0.25 and 8 mg/l, while the MIC₅₀ and MIC₉₀ for tetracycline were 0.25 and 16 mg/l, respectively. The MIC₅₀ and the MIC₉₀ were ≤ 0.06 and >64 mg/l, respectively, for erythromycin, tylosin and clindamycin. This resistance pattern indicated that some of these *A. pyogenes* isolates may carry an MLS_B resistance determinant. *A. pyogenes* isolates (12.5%) were resistant to erythromycin, and this percentage doubled when MICs were performed following induction with erythromycin. Of the 48 *A. pyogenes* isolates, 25 and 41.7% were resistant to MLS_B antimicrobial agents and the tetracycline derivatives, respectively. MLS_B resistance was present in 22.2 and 35.3% of *A. pyogenes* isolates of bovine ($n = 27$) or porcine ($n = 17$) origin. In contrast, 70.6% of porcine isolates were resistant to the tetracyclines, compared with 25.9% of bovine isolates. These data suggest that a large proportion of *A. pyogenes* field isolates may be resistant to these commonly used antimicrobial agents. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Arcanobacterium pyogenes*; Antibiotics; Susceptibility; Cattle-bacteria; Pig-bacteria

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1. Introduction

Arcanobacterium pyogenes is a common inhabitant of the mucous membranes of ruminants, pigs and other domestic animals (Carter and Chengappa, 1991). However, *A. pyogenes* is also a pathogen associated with liver abscesses in feedlot cattle (Lechtenberg et al., 1988; Nagaraja et al., 1996), pneumonia and septic arthritis in pigs (Høie et al., 1991; Hariharan et al., 1992), as well as with a variety of other opportunistic infections in these species.

Most cattle feedlots and swine operations in the US routinely use antibiotics as feed additives for growth promotion or disease prophylaxis. In fact, 83.2% of cattle feedlots and 92.7% of swine operations in the US use antibiotics, primarily tetracycline derivatives and tylosin, as feed or water additives (USDA, 1996, 2000). Specifically, tylosin is used to reduce the incidence of liver abscesses in feedlot cattle. *Fusobacterium necrophorum* is the primary etiologic agent of bovine liver abscesses (Scanlan and Hathcock, 1983), but *A. pyogenes* is the second most commonly isolated bacterium (Berg and Scanlan, 1982; Lechtenberg et al., 1988), being found in 10–90% of cases (Narayanan et al., 1998b; Nagaraja et al., 1999).

Few MIC determinations for *A. pyogenes* have been reported, mostly with European (Guérin-Faubleé et al., 1993; Jousimies-Somer et al., 1996) or Japanese (Yoshimura et al., 2000) isolates. This paper reports the antimicrobial susceptibility of 48, predominantly North American, *A. pyogenes* isolates to tetracyclines, macrolides and lincosamides, and whether these resistance determinants are inducible.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The *A. pyogenes* strains used in this study were field isolates obtained from veterinary diagnostic laboratories or personal collections predominantly from North America. These strains were isolated from cattle ($n = 27$), swine ($n = 17$), dogs ($n = 2$), a cat ($n = 1$) and a macaw ($n = 1$). *A. pyogenes* isolates were grown on brain heart infusion (BHI, Difco) agar plates, containing 5% bovine blood at 38 °C and 5% CO₂ for 48 h.

2.2. Antimicrobial agents

Chlortetracycline, oxytetracycline, tetracycline, clindamycin, erythromycin and tylosin (Sigma) were prepared according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (NCCLS, 1999).

2.3. Determination of MIC values

As there is no NCCLS method for the determination of MICs of *A. pyogenes*, a method based on that for *S. pneumoniae* was used (NCCLS, 1999). The inoculum was prepared by resuspending several isolated colonies in sterile Mueller–Hinton broth (Difco) containing

2% lysed, defibrinated horse blood (BBL Microbiology Systems) (MHB-LHB) and the suspension was adjusted to $OD_{600} = 0.1$ and then diluted 1/100 with MHB-LHB (approximately 1×10^6 CFU/ml). The antimicrobial agents to be tested were diluted in a doubling dilution pattern over the range 0.06–64 mg/l in the wells of sterile, 96-well, round bottom microtiter plates in 50 μ l volumes. The 50 μ l volume of inoculum was dispensed into each well, and the plates were sealed and incubated at 38 °C for 24 h. The MIC was read visually as the lowest concentration of the antimicrobial agent to prevent growth (turbidity), compared with the control (no antimicrobial agent added). Each isolate was tested three times in duplicate, and the endpoints for each antimicrobial agent did not differ by more than one dilution.

In order to determine MICs following induction, the *A. pyogenes* isolates were grown on BHI agar containing 5% bovine blood and 1 mg/l of the appropriate antimicrobial agent, prior to MIC determination, as described above.

3. Results

The fastidious nature of *A. pyogenes* required the addition of 2% LHB to MHB for optimal bacterial growth. In addition, incubation at 35 °C did not result in sufficient growth of *A. pyogenes* in the control wells (no antimicrobial agent added) within 24 h. Therefore, all MICs were performed at 38 °C and the results were determined after 24 h.

The MICs for two macrolides, erythromycin and tylosin, and a lincosamide, clindamycin, were determined in the absence of induction. The MIC₅₀ and MIC₉₀ for erythromycin, tylosin and clindamycin were ≤ 0.06 and >64 mg/l, respectively (Table 1). Assuming that an MIC of ≥ 8 mg/l indicates a resistant phenotype (NCCLS, 1999), 12.5% of *A. pyogenes* isolates were resistant to erythromycin, 20.8% of isolates were resistant to tylosin, and 25.0% of isolates were resistant to clindamycin.

Eight *A. pyogenes* isolates with MICs ≥ 0.5 –8 mg/l were subjected to induction with either erythromycin or tylosin prior to determination of their MIC (Table 2). With the exception of strain OX2, each of these isolates was induced to higher MICs with either erythromycin or tylosin, and induction with either antimicrobial agent resulted in the same increase in MIC (Table 2). OX2 was resistant to erythromycin and clindamycin, but was

Table 1
MICs of macrolides, lincosamides and tetracyclines for 48 *A. pyogenes* strains

Antimicrobial agent	Number of strains with MIC (mg/l)											
	0.06 ^a	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
Erythromycin	36	–	–	–	1	2	3	1	–	–	–	5
Tylosin	36	–	–	1	–	1	–	1	–	–	–	9
Clindamycin	36	–	–	–	–	–	–	1	–	–	–	11
Chlortetracycline	22	6	–	–	–	–	10	9	–	1	–	–
Oxytetracycline	6	15	3	1	1	2	5	11	3	1	–	–
Tetracycline	9	12	4	–	3	–	1	12	7	–	–	–

^a Indicates an endpoint of \leq for the MIC indicated.

Table 2

MICs of macrolide and lincosamide antibiotics for 48 *A. pyogenes* strains performed following induction

Strain	MIC for erythromycin		MIC for tylosin		MIC for clindamycin	
	None	Erythromycin or tylosin	None	Erythromycin or tylosin	None	Erythromycin or tylosin
OX2	>64	– ^a	0.5	0.5	8	8
E1DE	4	16	>64	–	>64	–
4759	4	16	>64	–	>64	–
3	2	>64	>64	–	>64	–
4	4	>64	>64	–	>64	–
B167	2	16	>64	–	>64	–
98-4277-2	1	>64	2	64	>64	–
52785-99	8	>64	8	>64	>64	–

^a Not done.

susceptible to tylosin (Table 2). However, the MIC of clindamycin for OX2 (8 mg/l), was much lower than for the other clindamycin resistant strains, while the MIC of tylosin (0.5 mg/l) was higher than for the majority of susceptible isolates. This data suggests that this strain probably carries a different resistance determinant than the other resistant *A. pyogenes* isolates. Higher MICs for this strain could not be induced by erythromycin, tylosin (Table 2) or clindamycin (data not shown). Therefore, the percentage of *A. pyogenes* isolates resistant to erythromycin (i.e., MIC \geq 8 mg/l) doubled from 12.5 to 25.0%, when MICs were performed following induction.

Resistance of these *A. pyogenes* isolates to erythromycin, tylosin and clindamycin indicated that the mechanism of resistance may be due to an MLS_B determinant. The 12 resistant *A. pyogenes* isolates were grown on BHI-blood agar containing 10 mg/l oleandomycin, spiramycin (macrolides) or lincomycin (lincosamide). All the isolates grew well in the presence of these drugs, indicating that the putative MLS_B determinant could potentially encode resistance to a wide range of macrolides and lincosamides.

The MICs of chlortetracycline, oxytetracycline and tetracycline were determined for the 48 *A. pyogenes* isolates. The MIC₅₀ and MIC₉₀ for chlortetracycline were 0.12 and 8 mg/l, respectively (Table 1). Similarly, the MIC₅₀ and MIC₉₀ for oxytetracycline were 0.25 and 8 mg/l, while the MIC₅₀ and MIC₉₀ for tetracycline were 0.25 and 16 mg/l, respectively. As some tetracycline resistance determinants are inducible, strains with MICs \geq 0.5–8 mg/l were induced with tetracycline prior to determination of their MICs. No increase in MICs was observed for any strain (data not shown), indicating that the tetracycline resistance was not inducible. Assuming an MIC of \geq 4 mg/l indicates a resistant phenotype, 41.7% of isolates were resistant to all three tetracycline derivatives, and it was the same 20 *A. pyogenes* isolates in every case. While NCCLS guidelines define \geq 16 mg/l tetracycline, chlortetracycline or oxytetracycline as the breakpoint for resistance (NCCLS, 1999), an MIC \geq 4 mg/l correlates with the presence of a known tetracycline resistance determinant, Tet W, in these strains (S.J. Billington and B.H. Jost, unpublished data). When the 20 resistant *A. pyogenes* isolates were grown on BHI-blood agar containing 10 mg/l demeclocycline, doxycycline or minocycline, all grew well, indicating that the Tet W determinant probably encodes resistance to all the tetracycline derivatives tested.

The MLS_B resistant *A. pyogenes* isolates were approximately equally distributed among isolates of bovine ($n = 27$) and porcine ($n = 17$) origin, with 22.2 and 35.3% resistant, respectively. However, tetracycline resistance was preferentially associated with porcine isolates, with 70.6% of isolates resistant to the tetracyclines, compared with only 25.9% of *A. pyogenes* isolates of bovine origin. Canine ($n = 2$), feline ($n = 1$) and avian ($n = 1$) isolates were not resistant to the MLS_B antimicrobial agents, and only the avian isolate was resistant to the tetracyclines.

4. Discussion

The development of antimicrobial resistance in normal flora, opportunistic pathogens such as *A. pyogenes* is of significance, not only for treatment of *A. pyogenes* infections, but also because normal flora can act as reservoirs of transferable antimicrobial resistances. Furthermore, the finding that the use of tylosin as a feed additive increased the percentage of liver abscesses containing *A. pyogenes* from 10 to 53% (Nagaraja et al., 1999), probably indicates that selection of tylosin-resistant *A. pyogenes* does occur in vivo.

Identification of the presence of transferable resistances contained on transposons and/or plasmids, requires that we first determine the type and prevalence of antimicrobial resistances in *A. pyogenes*. Tylosin resistance in *A. pyogenes* is most probably due to the presence of an MLS_B determinant, which encodes resistance to macrolides, lincosamides and streptogramin B antimicrobial agents. MLS_B resistance can be expressed in a constitutive or an inducible manner, and use of conventional MIC methodology may categorize strains with an inducible resistance as having a susceptible or intermediate phenotype. In fact, half of the erythromycin-resistant isolates identified in this study had an inducible phenotype, which would be characterized as susceptible or intermediate if tested in the absence of induction.

There are potentially three different MLS_B determinants present in *A. pyogenes*, the constitutively expressed determinant found in four isolates, the inducible determinant found in seven isolates, and the determinant found in OX2. Similarly, Guérin-Faublée et al. (1993) suggested that the erythromycin-resistant *A. pyogenes* isolates also carried an MLS_B resistance determinant, as these strains were also resistant to spiramycin, lincomycin and pristinamycin component I, a streptogramin B. Unfortunately, due to the unavailability of streptogramin B antimicrobial agents, we were unable to determine MICs against this class of drug. However, all the 48 *A. pyogenes* isolates were susceptible to virginiamycin, a mixture of streptogramin A and streptogramin B (data not shown), probably as a result of sensitivity to the virginiamycin M (streptogramin A) component.

US studies of *A. pyogenes* strains isolated from bovine liver abscesses concluded that no resistance to tylosin was found in these isolates (Narayanan et al., 1998a; Nagaraja et al., 1999), regardless of whether tylosin was used as a feed additive. In at least one of these studies, the data may have been misinterpreted, as at least 10% of *A. pyogenes* isolates tested had MICs > 12.5 mg/l (Narayanan et al., 1998a).

Resistance to the tetracyclines was widely present in the 48 *A. pyogenes* isolates tested, with 41.7% of isolates resistant to chlortetracycline, oxytetracycline and tetracycline, due to the presence of a non-inducible Tet W determinant (S.J. Billington and B.H. Jost,

unpublished data). Similar results have been found in other studies with 61.2–67.0% of *A. pyogenes* isolates resistant to tetracyclines (Guérin-Faublée et al., 1993; Yoshimura et al., 2000). These figures may reflect the long-term and widespread use of the tetracyclines in agriculture. Interestingly, while MLS_B resistance was more evenly distributed amongst bovine and porcine isolates, at 22.2 and 35.3%, respectively, tetracycline resistance was more predominant in porcine isolates (70.6%) compared with bovine isolates (25.9%). A similar observation was made by Yoshimura et al. (2000), where 85.7% of porcine, but only 57.1% of bovine *A. pyogenes* isolates were resistant to oxytetracycline. One possible reason for the disparity between isolates of different origin may be the different uses for tetracycline derivatives in cattle and swine. Tetracyclines are fed on a long-term basis for growth promotion in swine (USDA, 1996), whereas they are used for shorter periods in cattle, for the prevention of certain diseases upon entry into the feedlot (USDA, 2000).

These findings suggest that resistance of *A. pyogenes* to antimicrobial agents commonly used as feed and water additives in US agriculture is prevalent. Furthermore, the determinants encoding MLS_B resistance in *A. pyogenes* are not identical, and include constitutively expressed and inducible types. The presence of inducible resistance in some *A. pyogenes* isolates may lead to their categorization as intermediate or susceptible, resulting in the underestimation of MLS_B resistance in this organism. Further work characterizing the types of tetracycline and MLS_B resistance determinants in *A. pyogenes* is currently underway.

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