Research in Veterinary Science 94 (2013) 205–208

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Relationship between serotype and the antimicrobial susceptibility of *Mannheimia haemolytica* isolates collected between 1991 and 2010

K. Katsuda *, M. Kohmoto, O. Mikami

Tohoku Research Station, Viral Disease and Epidemiology Research Division, National Institute of Animal Health, NARO, 31 Uminai, Shichinohe, Kamikita, Aomori 039-2586, Japan

ARTICLE INFO

Article history:
Received 10 February 2012
Accepted 15 September 2012

Keywords:
Antimicrobial susceptibility
Calves
Mannheimia haemolytica
Respiratory disease
Serotype

ABSTRACT

The antimicrobial susceptibilities and serotype distribution of 310 *Mannheimia haemolytica* isolates obtained from cattle with bovine respiratory disease during 2002–2010 were investigated. Of the 310 isolates, 198 (63.9%) were resistant to at least one of the 16 tested antimicrobial agents. The resistance rates for ampicillin, amoxicillin, dihydrostreptomycin, kanamycin, oxytetracycline, doxycycline, chloramphenicol, thiamphenicol, nalidixic acid, enrofloxacin, and danofloxacin were 20.3%, 14.5%, 43.5%, 23.5%, 24.8%, 21.9%, 23.2%, 23.9%, 47.1%, 18.7%, and 18.7%, respectively. Almost 90% of the isolates belonged to three serotypes (serotypes A1, A2, and A6), and the relative prevalence of serotype A6 increased significantly over the last decade. Compared with bacteria belonging to other serotypes, bacteria belonging to serotype A6 exhibited a significantly higher antimicrobial resistance rates ($\chi 2$ test, $p < 0.05$).

The results of this investigation provide useful information for understanding the serotype prevalence and antimicrobial resistance patterns of one of the major bacteriological agents implicated in pneumonic pasteurellosis.

Mannheimia haemolytica is consistently detected as a complicating agent in bovine respiratory disease (BRD) and is recognised as an important pathogen in feedlots (Rice et al., 2007). Antimicrobials remain effective tools for the control of infections caused by this bacterium. In recent years, however, the testing of *M. haemolytica* isolates from cattle with BRD has revealed that an increasing proportion of isolates are resistant to antimicrobials (Esaki et al., 2005; Schwarz et al., 2004; Shin et al., 2005). Previously, we reported that resistance to antimicrobial agents was frequently observed among *M. haemolytica* isolates (Katsuda et al., 2009). Continuous monitoring of the susceptibility to antimicrobial agents and the serotype distribution is necessary to determine the current susceptibility status and to provide useful information for the prevention of pneumonic pasteurellosis in cattle. In this study, we investigated the serotype distribution and the levels of antimicrobial susceptibility of *M. haemolytica* isolates collected from cattle affected with pneumonic pasteurellosis.

A total of 310 *M. haemolytica* field isolates collected from lung lesion of cattle with pneumonic pasteurellosis in 31 prefectures were obtained between 2002 and 2010 in Japan. One isolate was collected per herd. All of the isolates were identified by standard protocols and were further characterised using API micro standardised strips (API 20NE; bioMérieux Japan Ltd., Tokyo, Japan) and PCR (Alexander et al., 2008). All of the isolates were stored in 1 ml of tryptic soy broth (Becton, Dickinson and Company, USA) supplemented with 20% glycerol (vol/vol) at −80 °C. Prior to serotyping and minimum inhibitory concentration (MIC) determination, the isolates were cultured on tryptic soy agar plates supplemented with 5% sheep blood.

To determine the susceptibility of the *M. haemolytica* isolates to 16 selected antimicrobial agents (ampicillin (ABPC), amoxicillin (AMPC), dihydrostreptomycin (SM), kanamycin (KM), oxytetracycline (OTC), doxycycline (DOXY), chloramphenicol (CP), thiamphenicol (TP), florfenicol (FF), nalidixic acid (NA), enrofloxacin (ERFX), danofloxacin (DNFX), colistin (CL), cefazolin (CEZ), ceftiofur (CTF), and cefquinome (CQN)), the agar dilution method (dilution range: 0.125–512.0 μg/mL) recommended by the Clinical and Laboratory Standards Institute subcommittee on Veterinary Antimicrobial Susceptibility Testing was used (Clinical and Laboratory Standards Institute, 2005). The following quality control strains were also tested: *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853. Additionally, *M. haemolytica* NCTC 9380 was included in the majority of the assays. The cut-offs used for CTF, KM, AMPC, CEZ, CTF, ERFX, and FF were adopted from the CLSI recommendations. For the remaining antimicrobials, the midpoint of the MIC peaks was defined as the resistance cut-off when the MIC distribution of the antimicrobials was bimodal.

Isolates were serotyped using the indirect haemagglutination (IHA) test (Biberstein, 1978) with *M. haemolytica* serotype-specific

* Corresponding author. Tel.: +81 176 62 5115; fax: +81 176 62 5117.
E-mail address: katsuda@affrc.go.jp (K. Katsuda).

0034-5288/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.rvsc.2012.09.015

Table 1
Antimicrobial susceptibility of *M. haemolytica* isolated from cattle with BRD.

Agents	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Break point[a] (μg/ml)	Number of resistant isolates (%)
Ampicillin (ABPC)	0.25–>512	2.0	128.0	8.0	63 (20.3)
Amoxicillin (AMPC)	⩽0.125–512.0	0.25	64.0	2.0	45 (14.5)
Dihydrostreptomycin (SM)	1.0–>512.0	16.0	>512.0	64.0	135 (43.5)
Kanamycin (KM)	2.0–>512.0	8.0	>512.0	64.0	73 (23.5)
Colistin (CL)	⩽0.125–2.0	0.25	0.5	-	0 (0.0)
Oxytetracycline (OTC)	0.25–512.0	1.0	32.0	8.0	77 (24.8)
Doxycycline (DOXY)	0.125–16.0	1.0	8.0	4.0	68 (21.9)
Chloramphenicol (CP)	0.25–128.0	1.0	64.0	32.0	72 (23.2)
Thiamphenicol (TP)	0.5–>512.0	1.0	256.0	16.0	74 (23.9)
Florfenicol (FF)	0.25–32.0	1.0	2.0	32.0	1 (0.3)
Cefazolin (CEZ)	0.125–16.0	1.0	4.0	–	0 (0.0)
Ceftiofur (CTF)	⩽0.125–0.125	⩽0.125	⩽0.125	–	0 (0.0)
Cefquinome (CQN)	⩽0.125–0.125	⩽0.125	⩽0.125	–	0 (0.0)
Nalidixic acid (NA)	1.0–512.0	4.0	256.0	32.0	146 (47.1)
Enrofloxacin (ERFX)	⩽0.125–16.0	⩽0.125	8.0	4.0	58 (18.7)
Danofloxacin (DNFX)	⩽0.125–16.0	⩽0.125	16	4.0	58 (18.7)

[a] The cut-offs used for CTF, KM, AMPC, CEZ, CTF, ERFX, and FF were adopted from the CLSI recommendations. For the remaining antimicrobials, the midpoint of the MIC peaks was defined as the resistance cut-off when the MIC distribution of the antimicrobials was bimodal.

antisera (Katsuda et al., 2008). Antiserum against the newly described serotype 17 was not included in the test. Statistical analysis was performed with GraphPad Prism® software, version 5.0 (MDF Co., Ltd. Tokyo Japan). The results were considered statistically significant if $p \leqslant 0.05$.

The MICs for 16 selected antimicrobial agents were determined, and the antimicrobial susceptibilities of the *M. haemolytica* isolates were tested (Table 1.). The MIC distributions of all antimicrobials, except CL and cephalosporin drugs, were bimodal. Of the 310 isolates, 198 (63.9%) were resistant to at least one of the 16 tested antimicrobial agents. The resistance rates for ABPC, AMPC, SM, KM, OTC, DOXY, CP, TP, NA, ERFX, and DNFX were 20.3%, 14.5%, 43.5%, 23.5%, 24.8%, 21.9%, 23.2%, 23.9%, 47.1%, 18.7%, and 18.7%, respectively. There was only one isolate resistant to FF (0.3%), and isolates resistant to CL and cephalosporin drugs (CEZ, CTF, and CQN) were not observed, thus indicating a high susceptibility of this bacterium to these agents. We also analysed the antimicrobial resistance rates of *M. haemolytica* isolates in this study compared to resistance rates published by Katsuda et al. (2009). Using these previous data, we compared the patterns of resistance to each class of antimicrobial agents of 480 *M. haemolytica* isolates collected between 1991 and 2010. Of the 480 isolates, 310 isolates was collected newly in this investigation, and the rest of 170 isolates were already used in previous reports. We carried out MIC and serotype test of this 170 isolates again in this investigation blinded to the results from previous testing.

The antimicrobial agents used in this research belong to eight classes: penicillins, cephalosporins, chloramphenicols, tetracyclines, aminoglycosides, quinolones, fluoroquinolones, and peptides. Of the 81 isolates obtained before 2000, 50.6% exhibited susceptibility to all classes of antimicrobial agents used in this study, 34.6% were resistant to one class, and 14.8% were resistant to more than one antimicrobial class. The proportion of 2001–2005 recovered isolates resistant to a single antimicrobial was lower (20.0%) than during 1991–2000 ($\chi 2$ test, $p < 0.05$), while a significantly greater number of isolates (38.9%, $\chi 2$ test, $p < 0.05$) were resistant to more than one antimicrobial in comparison with 1991–2000.

Among the 234 isolates collected from 2006 to 2010, the percentage of isolates susceptible to all antimicrobials was lower (37.6%) than the percentage of susceptible isolates during 1991–2000 ($\chi 2$ test, $p < 0.05$). The proportion of isolates resistant to a single antimicrobial was 30.3%, and 32.1% of the isolates were resistant to more than one antimicrobial. A significant difference was observed in comparison with 1991–2000 ($\chi 2$ test, $p < 0.05$) (Fig. 1a).

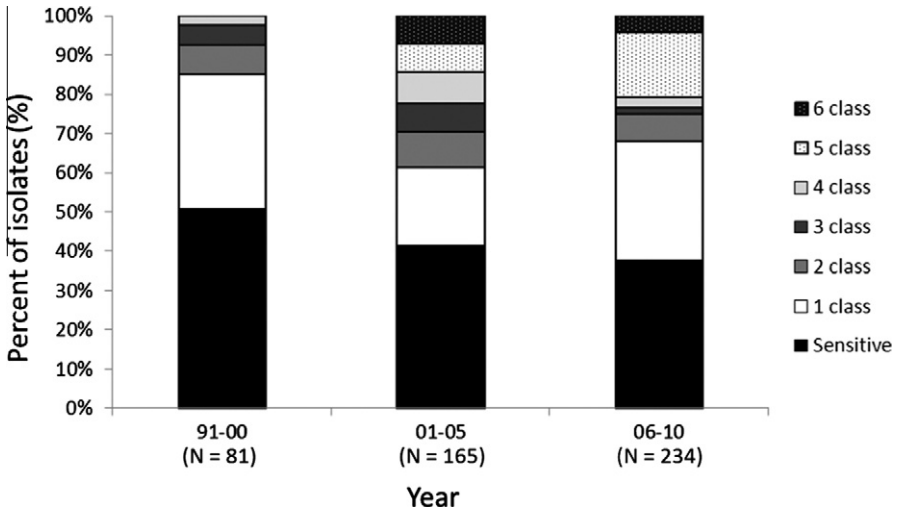

Fig. 1a. Trends in the antimicrobial resistance pattern of *M. haemolytica*. Sensitive: sensitive to all classes of antimicrobial agents used in this study; 1 class–6 classes: resistant to the indicated number of classes of antimicrobial agents.