Veterinary Microbiology 171 (2014) 232–235

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short Communication

Antimicrobial resistance in bacteria associated with porcine respiratory disease in Australia

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ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form 4 March 2014
Accepted 8 March 2014

Keywords:
Porcine respiratory disease
Antimicrobial susceptibility testing
Antimicrobial resistance

ABSTRACT

The porcine respiratory disease complex greatly affects the health and production of pigs. While antimicrobial agents are used to treat the respiratory infections caused by bacterial pathogens, there is no current information on antimicrobial resistance in Australian pig respiratory bacterial isolates. The aim of this study was to determine the antimicrobial resistance profiles, by determining the minimum inhibitory concentration of nine antimicrobial agents for 71 *Actinobacillus pleuropneumoniae*, 51 *Pasteurella multocida* and 18 *Bordetella bronchiseptica* cultured from Australian pigs. The majority of *A. pleuropneumoniae* isolates were resistant to erythromycin (89%) and tetracycline (75%). Resistance to ampicillin (8.5%), penicillin (8.5%) and tilmicosin (25%) was also identified. The *P. multocida* isolates exhibited resistance to co-trimoxazole (2%), florfenicol (2%), ampicillin (4%), penicillin (4%), erythromycin (14%) and tetracycline (28%). While all the *B. bronchiseptica* isolates showed resistance to beta-lactams (ampicillin, ceftiofur and penicillin), some were resistant to erythromycin (94%), florfenicol (6%), tilmicosin (22%) and tetracycline (39%). The incidence of multiple drug resistance (MDR) varied across the species – in *B. bronchiseptica*, 27.8% of resistant isolates showed MDR, while 9.1% of the resistant isolates in *A. pleuropneumoniae*, and 4.8% in *P. multocida* showed MDR. This study illustrated that Australian pig strains of bacterial respiratory pathogens exhibited low levels of resistance to antimicrobial agents commonly used in the pig industry.

1. Introduction

The porcine respiratory disease complex (PRDC), one of the most significant problems affecting health and production in the pig industry worldwide, is described as a multifactorial pneumonic state resulting from the interaction of bacteria, viruses and stresses caused by management, environment and genetic conditions (Opriessnig et al., 2011). A range of bacterial pathogens is associated with the initiation and progress of PRDC, with *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Pasteurella multocida* and *Bordetella bronchiseptica* having significant roles (Fablet et al., 2011; Opriessnig et al., 2011).

The use of antimicrobial agents, beta-lactams (ampicillin, penicillin and cephalosporins) (except for *B. bronchiseptica*), co-trimoxazole (sulfonamide and trimethoprim combination), florfenicol, macrolides (erythromycin, tilmicosin and tulathromycin) and tetracyclines remains the best treatment option to control PRDC (Karriker et al., 2013). The usage of antimicrobial agents has the potential to select for antimicrobial resistance (Barton et al., 2003).

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http://dx.doi.org/10.1016/j.vetmic.2014.03.014
0378-1135/

Resistance to antimicrobials commonly used to treat PRDC have been detected previously in porcine respiratory disease pathogens from many countries (Vicca et al., 2004; de la Fuente et al., 2007; San Millan et al., 2009; Tang et al., 2009; Chander et al., 2011; Kucerova et al., 2011; Nedbalcová and Kucerova, 2013).

In the past, antimicrobial resistance in Australia was reported in *A. pleuropneumoniae* (Eaves et al., 1989) and *P. multocida* (Stephens et al., 1995). However, no information exists for *B. bronchiseptica*. Thus, this study aimed to determine the antimicrobial susceptibility of *A. pleuropneumoniae*, *P. multocida* and *B. bronchiseptica* Australian isolates against antimicrobial agents used for bacterial respiratory pathogens.

2. Materials and methods

The bacterial isolates tested were obtained from Australian pigs in diagnostic disease investigations and then submitted to the Microbiology Research Group, EcoSciences Precinct, Department of Agriculture Fisheries and Forestry (DAFF), Queensland, Australia for confirmatory identification and/or serotyping. A total of 71 *A. pleuropneumoniae*, 51 *P. multocida* and 18 *B. bronchiseptica* isolates collected between the years 2002 and 2013 were selected from the culture collection of the Microbiology Research Group. All isolates were diagnostic submissions from Australian pig herds. The *A. pleuropneumoniae* isolates represent 19% of the total available culture collection of the Microbiology Research Group and originated from New South Wales (8 isolates), Queensland (24 isolates), South Australia (8 isolates), Victoria (24 isolates) and Western Australia (7 isolates). The *P. multocida* isolates came from New South Wales (12 isolates), Queensland (22 isolates), South Australia (3 isolates), Victoria (1 isolate) and Western Australia (13 isolates). The *B. bronchiseptica* isolates came from New South Wales (4 isolates), Queensland (13 isolates) and South Australia (1 isolate). All isolates of *P. multocida* and *B. bronchiseptica* existing in the culture collection were included in this study. With the exception of *B. bronchiseptica*, all the isolates had been previously identified by a relevant species specific polymerase chain reaction (Gram and Ahrens, 1998; Townsend et al., 1998; Miflin and Blackall, 2001). The *B. bronchiseptica* isolates had been previously identified by sequencing of the 16S rDNA gene using a previously described method (Blackall et al., 2001).

Antimicrobial resistance was detected by determination of MIC in duplicate using CLSI standards and recommendations (CLSI, 2013). The media used were chocolate Mueller Hinton agar (BD) for *A. pleuropneumoniae*; and cation adjusted Mueller Hinton broth (BD) for *P. multocida* and *B. bronchiseptica*. The antimicrobials used were ampicillin, ceftiofur, co-trimoxazole, florfenicol, erythromycin, penicillin, tetracycline, tilmicosin and tulathromycin. As per the CLSI (2013), the quality control strains used were *A. pleuropneumoniae* (ATCC 27090) and *S. aureus* (ATCC 29213).

The MIC was defined as the lowest antimicrobial concentration that inhibited bacterial growth. The interpretation of MIC of each antimicrobial agent against the three bacterial species was based on the breakpoints provided by the CLSI (2013), where available. As there are no CLSI interpretative breakpoints for penicillin, the one for ampicillin was used (CLSI, 2013). The breakpoints (shown in Table 1) for some antimicrobial agents were taken from other published studies and are detailed in the following text. For *A. pleuropneumoniae*, breakpoints for erythromycin and co-trimoxazole were the ones used by Archambault et al. (2012). For *P. multocida*, the breakpoints used were from the CLSI guidelines (CLSI, 2013) except for erythromycin (Tang et al., 2009) and co-trimoxazole (Archambault et al., 2012). The breakpoints used for *B. bronchiseptica* were the values provided by the CLSI guidelines (CLSI, 2013) where available while some were taken from the published literature – erythromycin (Tang et al., 2009) and co-trimoxazole (Archambault et al., 2012).

3. Results and discussion

The MIC distribution of 71 *A. pleuropneumoniae*, 51 *P. multocida* and 18 *B. bronchiseptica* isolates, the percentage of resistance in each antimicrobial as well as the MIC_{50} and MIC_{90} are shown in Table 1.The MICs of the reference strains in each test run were within the CLSI acceptable quality control ranges. All *A. pleuropneumoniae* were susceptible to ceftiofur, co-trimoxazole, florfenicol and tulathromycin. Overall, 66 of 71 (93%) of the *A. pleuropneumoniae* isolates were resistant to one or more antimicrobials, showing seven antimicrobial resistance patterns. Resistance to ampicillin (8.5%), penicillin (8.5%), tilmicosin (25%), tetracycline (75%) and erythromycin (89%) was detected. All *P. multocida* isolates were susceptible to ceftiofur, tilmicosin and tulathromycin. Twenty-one (41%) of the isolates exhibited resistance, showing five antimicrobial resistance patterns in which 2% were resistant to co-trimoxazole, 2% to florfenicol 4% to ampicillin and penicillin, 14% to erythromycin and 28% to tetracycline. All *B. bronchiseptica* isolates were susceptible to co-trimoxazole and tulathromycin and resistant to all beta-lactams included in this study. The obtained MICs showed resistance to florfenicol (6%), tilmicosin (22%), tetracycline (39%) and erythromycin (94%). The antimicrobial resistance of *B. bronchiseptica* isolates demonstrated six patterns.

In examining the results of the current study, there are a number of issues that need to be considered. Firstly, it is important to understand that the study is based on a collection of isolates submitted for identification and/or serotyping from across Australia. The collection, however, cannot be regarded as being representative of the full diversity of these pathogens present in the Australian pig herd. A much larger study, seen for example in the recent North American study by Portis et al. (2013), would be required to gain insight into the national picture in Australia. Secondly, while there is no specific knowledge, it is highly likely that the isolates used in the current study would have come from pigs exposed to antimicrobial treatment. Indeed, the antimicrobial agents used in this study are all registered for use in Australian pigs (https://portal.apvma.gov.au/pubcris). The VetPath program in Europe (de Jong et al., 2012) is seeking to address this issue by examining isolates obtained prior to the commencement of any antimicrobial treatment program.