In-vitro activity of ceftiofur against Australian isolates of the family *Pasteurellaceae* associated with respiratory disease in cattle and pigs

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Ceftiofur is a broad spectrum, b-lactamase-resistant cephalosporin (Yancey et al 1987). Studies performed outside Australia have established that ceftiofur inhibits the growth of isolates of Pasteurella multocida, Pasteurella haemolytica, Actinobacillus pleuropneumoniae, Haemophilus somnus, Escherichia coli and Salmonella typhimurium at concentrations of 0.25mg/ml or less (Yancey et al 1987; Post et al 1991; Watts et al 1994).

A field study in Canada has demonstrated that ceftiofur is effective in the treatment of natural outbreaks of respiratory disease in feedlot cattle proving superior to trimethoprim-sulfadoxine (Jim *et al* 1992). In that study, cattle receiving ceftiofur had a significantly lower percentage of animals requiring more than three days of treatment for the initial treatment episode compared with the cattle receiving trimethoprim-sulfadoxine.

In this paper, we report on the *in vitro* antibacterial activity of ceftiofur against isolates of *P multocida* and *P haemolytica* (19 and 34 respectively) obtained from Australian cattle and isolates of *P multocida* and *A pleuropneumoniae* (30 and 50 respectively) from Australian pigs. The *Pasteurella* isolates were selected from as diverse a background as possible with isolates from New South Wales (1 *P haemolytica*, 11 bovine *P multocida* and 10 porcine *P multocida* isolates), Queensland (33 *P haemolytica*, 7 bovine *P multocida* and 11 porcine *P multocida* isolates) and Victoria (1 bovine *P multocida* isolate and 9 porcine *P multocida* isolates). The isolates of *A pleuropneumoniae* examined were selected to represent the known genetic diversity of this species in Australia (Hampson *et al* 1993) and included all 7 serovars known to occur in Australia (Hampson *et al* 1993; Blackall and Pahoff 1995). Representatives of the currently unserotypable isolates were also included (Blackall and Pahoff 1995). A reference strain of *E. coli* (ATCC25922) was used as a control strain.

The minimal inhibitory concentration (MIC) of ceftiofur* for the isolates was determined by a standardised agar dilution method as described previously (Stephens *et al* 1993) with some modifications. Firstly, the antibiotic plates did not contain lysed horse blood. Secondly, for the *A pleuropneumoniae* isolates, all media were supplemented with 0.0024% (w/v) reduced nicotinamide adenine dinucleotide (NADH). The agar plates contained a final concentration of ceftiofur that varied from 50 mg/ml to 0.20 mg/ml. The test organisms were grown overnight in tryptose phosphate broth† for the *Pasteurella* spp or tryptose phosphate broth† plus 0.0025% NADH for *A pleuropneumoniae* and were diluted 10^{-3}, to yield about 5×10^5 colony forming units/mL (Blackall *et al* 1995). One l µL of this dilution was then inoculated onto the plates. A plate that did not contain any antibiotic was included with each set of organisms. All organisms were also plated onto 5% sheep blood agar to confirm the purity of the inoculum. Each plate was also inoculated with the control strain of *E coli* (ATCC25922). The inoculated plates were incubated aerobically at 37°C for 18 hours. The MIC was taken as the lowest concentration to prevent growth. The MIC_{95} was defined as that concentration of ceftiofur that inhibited 95% of the isolates examined.

The MIC for the reference strain of *E coli* was always 0.78 mg/mL. This value is within the expected range (0.25 - 1.0 mg/mL) for this strain (Anon 1990). The MICs for all the bovine and porcine *P multocida*, the bovine *P haemolytica* and the *A pleuropneumoniae* isolates were ≤ 0.2 mg/mL. As no isolate of any of the three species tested was capable of growing at the minimum antibiotic concentration used in this study (0.2 mg/mL), the MIC_{95} of ceftiofur for all the isolates examined in this study was ≤ 0.2 mg/mL. The recommended in vitro interpretative criteria for ceftiofur are as follows: growth at ≥ 8 mg/mL = resistant, growth at 4 mg/mL but no higher = intermediate sensitivity; growth at 2 mg/mL but no higher = susceptible On the basis of these figures, all 34 isolates of *P haemolytica*, all 49 isolates of *P multocida* and all 50 isolates of *A pleuropneumoniae* were susceptible to ceftiofur.

Similar studies, based on isolates of these species obtained from American animals, reported an MIC_{95} of ceftiofur for these three species of either ≤ 0.06 mg/ml (Yancey *et al* 1987; Watts *et al* 1994) or ≤ 0.125 mg/mL (Post *et al* 1991). The higher figure reported in our study is explained by the fact that the lowest antibiotic dilution used in our study was 0.2 mg/ml. In both our study and the three previous studies (Yancey *et al* 1987; Post *et al* 1991; Watts *et al* 1994), all isolates examined have been found to be susceptible to ceftiofur. The results of our study have provided evidence that ceftiofur is highly effective *in vitro* against Australian bovine and porcine isolates of *Pasteurella* spp and porcine isolates of *A pleuropneumoniae*.

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* Ceftiofur sodium, Lot No. 555-1212, Upjohn Company, Kalamazoo, USA

† Oxoid CM283, Oxoid Australia Pty Ltd, West Heidelberg, Vic