APRAMYCIN, COLISTIN, NEOMYCIN AND PARAMOMYCIN MIC DISTRIBUTION FROM CLINICAL ISOLATES OF *KLEBSIELLA PNEUMONIAE*

Saggiorato M.[1]*, Scandurra S.[1], Pradella G.[1], Bacchin C.[2], Guolo A.[2], Agnoletti F.[2]

[1]ELI LILLY Italia S.p.A., Divisione Elanco Animal Health, Via Gramsci 733, Sesto Fiorentino (FI), Italy
[2]Istituto Zooprofilattico Sperimentale delle Venezie, Viale Brigata Treviso 13/a, 31100 Treviso, Italy
*Corresponding author: saggiorato_marco@lilly.com

ABSTRACT

Antimicrobial therapy continues to be important in reducing losses due to enteric forms of *Klebsiella pneumoniae* subsp. *pneumoniae* (*K. pneumoniae*) disease in rabbit intensive farms, in which this bacterium is frequently isolated from the gastrointestinal tract of suckling rabbits, between the 2nd and 4th week of age, showing a case history of diarrhoea. Commonly *K. pneumoniae* is characterized by a high resistance to the antimicrobials and for this reason is important to have up to date information in order to define a precision therapy according to principles of antibiotics judicious use guidelines. Although the enteric forms caused by *K. pneumoniae* diseases have been documented as frequent and economically important in France, Spain and Italy, there are no published reports on the antimicrobial activity of approved compounds against Italian strains. In this study, the authors report the activity of 4 different antimicrobials against 32 recovered isolates of *K. pneumoniae*. These isolates represent accessions from 2 geographic regions of the North-eastern Italy (Veneto and Friuli Venezia Giulia) where the rabbit breeding represent a widespread zootechnical practice. The minimum inhibitory concentration (MIC) values were determined by agar dilution according to the protocol proposed by NCCLS/CLSI (M31-A2 manual, 2004). MIC_{50}, MIC_{90}, geometric mean were calculated, and the values used for comparisons. Resulted MICs were: apramycin MIC_{50} 2 µg/ml, MIC_{90}>256 µg/ml; colistin MIC_{50} 32 µg/ml, MIC_{90} 64 µg/ml; neomycin MIC_{50} 2 µg/ml, MIC_{90} 128 µg/ml; paramomycin MIC_{50} 2 µg/ml, MIC_{90}>256 µg/ml. The geometric means of the MIC values obtained for apramycin and neomycin were 6.04 µg/ml and 6.58 µg/ml respectively, lower then those obtained for paramomycin (10.60 µg/ml) and colistin (24.67 µg/ml); however, among examined antimicrobials, apramycin, with the 78.1% of bacterial strains that present low MIC values (≤4 µg/ml), demonstrated the highest *in vitro* pharmacological activity against *K. pneumoniae.*

Key words: *Klebsiella pneumoniae*, Antimicrobial, Minimum Inhibitory Concentration (MIC), Rabbit.

INTRODUCTION

Antimicrobial therapy continues to be important in reducing losses due to enteric forms of *Klebsiella pneumoniae* subsp. *pneumoniae* (*K. pneumoniae*) disease in rabbit intensive farms, in which this bacterium is frequently isolated from the gastrointestinal tract of suckling rabbits, between the 2nd and 4th weeks of age, showing a case history of diarrhoea (Boucher and Nouaille, 1996).

The digestive disease is the main cause of mortality in industrial fattening rabbit farms. In a recent publication (Morel-Saives *et al.*, 2007), the economical impact of an episode of digestive disease was evaluated to be 0.78 € by produced rabbit. This cost allowed us to look for a therapeutic strategy. Although the enteric forms caused by *K. pneumoniae* diseases have been documented as frequent and economically important in France, Spain and Italy, there are no published reports on the antimicrobial activity of approved compounds against Italian strains. Commonly *K. pneumoniae* is characterized by a high resistance to the antimicrobials (Babini and Livermore, 2000) and for this reason it is important

to have up to date information in order to define a precision therapy according to principles of antibiotics judicious use guidelines (WHO, 2003; OIE, 2004).

Apramycin is an aminoglycoside antibiotic produced by *Streptomyces tenebrarius* structurally unique and that contains a monosubstituted deoxystreptamine. Colistin (polymyxin E) is a polymyxin antibiotic produced by certain strains of *Bacillus polymyxa* var. *colistinus*. Neomycin is an aminoglycoside antibiotic produced by *Streptomyces fradiae*. Paramomycin is a broad spectrum aminoglycoside antibiotic produced by *Streptomyces riomosus* var. *paramomycinus*. All these different antimicrobials are effective against Gram negative bacteria.

In this study, the authors report the activity of these 4 antimicrobials against 32 recovered isolates of *K. pneumoniae.* These isolates represent accessions from Italian regions where the rabbit breeding represent a widespread zootechnical practice. The objective of this study was to assess the good *in vitro* pharmacological activity against *K. pneumoniae* of apramycin compared to colistin, neomycin and paramomycin and consequently corroborate its therapeutic usefulness to control the enteric forms caused by *K. pneumoniae* in rabbit intensive farms according to principles of antibiotics judicious use guidelines and to the concept of precision therapy (Pradella *et al.*, 2007).

MATERIALS AND METHODS

MIC is defined as the lowest concentration of antimicrobial substance which, under defined *in vitro* conditions, prevents the growth of bacteria. First and foremost it should be noted that currently there are no CLSI (previously NCCLS) interpretive criteria available for apramycin, colistin, neomycin and paramomycin. In this study, a total of 32 strains of *K. pneumoniae* recovered from rabbits showing clinical signs of enteric forms caused by *K. pneumoniae* in Veneto (n=22) and Friuli Venezia Giulia (n=10) were examined. The strains were collected from diverse areas of each region, geographically distant farms and not from the same outbreak, as recommended by the CVMP627 guidelines. All the strains were stored at a nominal temperature of -80°C in Cryobank tubes until the moment of the test execution.

The following antimicrobial agents were used: apramycin (supplied by ELANCO), colistin sulfate (Sigma), neomycin trisulfate hydrate (Sigma) and paramomycin sulfate (USP).

The instructions provided in the respective analysis certificates were followed to solubilize the standard powders.

The test system was a standardized agar dilution MIC methodology, as described by the Clinical Laboratory Standards Institute (CLSI – previously NCCLS). To ensure compliance with CLSI recommendations, randomly selected standardized bacterial inocula used for each MIC test were enumerated. Antibiotic dilutions and susceptibility testing was done as described in CLSI M31-A2 documents.

To monitor performance and reproducibility of the MIC test, *Klebsiella pneumoniae* ATCC 700630 and *Escherichia coli* ATCC 25922 were used.

Reproducibility of MIC methodology was monitored on the basis of results obtained against the control strains. During a series of experiments in which consistent test conditions (culture medium, incubation conditions and preparation of antimicrobial agent) are used, the MIC result obtained for a single test compound against a given control strain should not vary by more than ±1 doubling dilution either side of the median value. This was followed by the calculation of the MIC_{50}, MIC_{90}, the geometric mean of the MICs and the MIC range. All MIC values were expressed in µl/ml.

RESULTS AND DISCUSSIONS

The MICs of the 32 *K. pneumoniae* strains, are summarized in Table 1 and the quality control (QC) strains results are provided in Table 2. The QC strains consistently gave reproducible results thus validating the test system. In Table 3 are summarized MIC value data, indicating MIC_{50}, MIC_{90}, geometric mean and MIC range. To calculate the MIC_{90}, the MIC_{50} and the geometric mean, the MIC values >256 µg/ml were approximated to 256 µg/ml.

Table 1: Summary of the apramycin, colistin, neomycin and paramomycin MIC distribution from clinical isolates of *K. pneumoniae*

	MIC (µg/ml)														
Antimicrobial	0.016	0.032	0.63	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Apramycin								21	4				1			6
Colistin						2				1	2	23	4			
Neomycin							15	2	1	1		3	6	3	1	
Paramomycin							13	6						1		12

Table 2: Summary of quality control strains MIC distribution

Antimicrobial	MIC (µg/ml) against *Escherichia coli* ATCC25922	MIC (µg/ml) against *Klebsiella pneumoniae* ATCC700630
Apramycin	4	2
Colistin	0.5	0.5
Neomycin	4	8
Paramomycin	4	64

Table 3: Summary of the antimicrobials activity against the *K. pneumoniae* strains

	MIC (µg/ml)			
Antimicrobial	MIC_{50}	MIC_{90}	Geometric Mean	MIC Ranges
Apramycin	2	>256	6.04	2 – 256
Colistin	32	64	24.67	0.5 - 64
Neomycin	2	128	6.58	1 – 256
Paramomycin	2	>256	10.60	1 – 256

The Minimum Inhibitory Concentration (MIC) of apramycin, colistin, neomycin and paramomycin against 32 strains of *K. pneumoniae* recovered from rabbits in Veneto e Friuli Venezia Giulia were determined using standardized CLSI methods. MIC_{50} and MIC_{90} of apramycin against *K. pneumoniae* strains were respectively 2 and >256 µg/ml. MIC_{50} and MIC_{90} of colistin against *K. pneumoniae* strains were respectively 32 and 64 µg/ml. MIC_{50} and MIC_{90} of neomycin against *K. pneumoniae* strains were respectively 2 and 128 µg/ml. MIC_{50} and MIC_{90} of paramomycin against *K. pneumoniae* strains were respectively 2 and >256 µg/ml.

The geometric means of the MIC values obtained for apramycin and neomycin were 6.04 µg/ml and 6.58 µg/ml respectively, lower then those obtained for paramomycin (10.60 µg/ml) and colistin (24.67 µg/ml). However, among examined antimicrobials, apramycin, with the 78.1% of bacterial strains that present low MIC values (≤4 µg/ml), demonstrated a good activity against strains of *K. pneumoniae* recovered from rabbits in Italy.

CONCLUSIONS

The MIC determination results demonstrate a highest *in vitro* pharmacological activity of apramycin compared to colistin, neomycin and paramomycin against *K. pneumoniae* of rabbit origin and clearly suggest its predictive therapeutic usefulness to control enteric forms of *Klebsiella pneumoniae* subsp. *pneumoniae* (*K. pneumoniae*) disease in rabbit intensive farms, according to principles of antibiotics judicious use guidelines and to the concept of precision therapy.

REFERENCES

Babini G.S., Livermore D.M. 2000. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997–1998. *J. Antimicrob. Chemother., 45, 183–189.*

Boucher S., Nouaille L. 1996. Manuel pratique des maladies des lapins. *France Agricole éd., Paris, 256*

Morel-Saives A., Limet A. 2007. Evaluation de l'impact technico-économique des maladies digestives chez le lapin d'engraissement; Intérêt de la mise en place d'un traitement. *In: Proc. 12èmes Journées de la Recherche Cunicole, 2007 November, Le Mans, France, 239-242.*

NCCLS. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard-second edition. NCCLS document M31-A2 (ISBN 1-56238-461-9). *NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2002.*

Pradella G., Scandurra S., Vecchi M. 2007. Strategie per l'ottimizzazione degli antibiotici in coniglicoltura: uso responsabile. *In: Proc. Giornate di Coniglicoltura ASIC, 2007 September, Forlì, Italy, 91-93.*

Office International des Epizooties 2004. Terrestrial Animal Health Code - 2003. Appendix 3.9.3: Guidelines for the responsible and prudent use of antimicrobial agents in veterinary medicine. *Available at: www.oie.int/eng/normes/MCode/A_00161.htm.*

World Health Organization 2003. WHO global principles for the containment of antimicrobial resistance in animals intended for food. Report of a WHO Consultation. *Available at www.who.int/emc/diseases/zoo/who_global_principles/index.htm.*