Occurrence and distribution of extended spectrum β-lactamase and AmpC-β-lactamase-producing Enterobacteriaceae and methicillin resistant *Staphylococcus aureus* in companion animals in New Zealand

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

In

Microbiology

Institute of Veterinary, Animal and Biomedical Sciences

Massey University

Palmerston North, New Zealand

Ali Karkaba

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http://www.bbc.com/news/health-34857015

http://www.stuff.co.nz/national/health/9418833/Science-under-siege-from-superbug

Some Alarming news during my first year in New Zealand

Abstract

The increasing incidence of infections with extended spectrum-lactamase (ESBL)- and AmpC-producing Enterobacteriaceae, and methicillin-resistant *Staphylococcus aureus* (MRSA) in humans in the last decade is a matter of concern. There is a paucity of data on the incidence of infections with these bacteria in animals, partly because veterinary diagnostic laboratories do not routinely test for these organisms in clinical specimens. The carriage rate of these bacteria by companion animals is also unknown.

This PhD project aimed to investigate the occurrence of ESBL/AmpC-producing Enterobacteriaceae and MRSA in clinical specimens from animals in New Zealand, and examine the carriage of multidrug-resistant (MDR), ESBL/AmpC-*E. coli*, and MRSA in cats and dogs in Auckland.

The results of this project indicate that ESBL/AmpC-producing *E. coli* and MRSA cause clinical infections in companion animals in New Zealand. The circulation of these bacteria is likely to be posing therapeutic challenges to unaware veterinarians. The bacteria causing infections or carried by companion animals are genetically similar to those found in humans in New Zealand, raising public health concerns about the role of carrier animals as potential sources of zoonotic infections.

General Introduction and overview of thesis

The relationship between humans and animals have changed, and pets are today considered an integral part of the family. However, this close proximity of pets is considered a potential hazard as a reservoir for antimicrobial resistant bacteria and potential zoonosis (Chomel 2011). Antimicrobial resistance is an emerging global problem, and New Zealand recognizes this threat in line with the rest of the world and is trying to reduce this burden using the 'One Health' approach (Gibbs 2014). This requires leadership and commitment from all sectors, in recognition that antimicrobial resistant bacteria abound in the different ecosystems shared by humans, food animals, and companion animals.

The widespread use and misuse of antimicrobials is considered the main factor driving the increase of antimicrobial resistance and the accelerated evolution and emergence of multi-drug resistant organisms that are difficult-to-treat (Alekshun and Levy 2006; Johns *et al.* 2012; Guo *et al.* 2015). The emergence of resistance to multiple drugs in animals decreases the efficacy of antimicrobials for the treatment of bacterial infections and may increase the rate of zoonotic resistant organisms (Yamamoto *et al.* 2014). *Escherichia coli* and *Staphylococcus aureus* are two of the most commonly isolated antimicrobial resistant bacteria in humans and animals (Yamamoto *et al.* 2014). Several studies have reported similar antimicrobial resistance profiles and genetic makeup in *E. coli* and *S. aureus* isolates recovered from both clinically affected and healthy humans and pets (Johnson *et al.* 2008; Davis *et al.* 2015; Guo *et al.* 2015; Carvalho *et al.* 2016; Loncaric *et al.* 2016).

The acquisition of antimicrobial resistance occurs mainly through mutation at the antimicrobial target sites, or by transfer of resistance genes via mobile elements, such as plasmids. In particular, a number of clinically important antimicrobial resistance mechanisms are mediated by the production of extended spectrum β-lactamases (ESBLs) or AmpC β-lactamases in Gram-negative bacteria, and by target site modification in the Gram-positive species *Staphylococcus aureus* (methicillin resistant *Staphylococcus aureus;* MRSA). Production of β-lactamase enzymes by infecting bacteria is

associated with therapy failure of β-lactam antimicrobials, prolonged and recurrent infections, and sometimes mortality (Hordijk *et al.* 2013). Furthermore, bacteria producing these enzymes are often also resistant to other antimicrobial classes, further complicating treatment (Jacoby 2005; Monecke *et al.* 2011; Bush 2012).

In New Zealand, as elsewhere, the incidence of clinical infections with ESBL and AmpC producing Enterobacteriaceae, in particular *E. coli*, and with MRSA, has increased significantly in humans in the last decade (Dyet *et al.* 2014; Heffernan and Bakker 2016). However, there is a paucity of data on antimicrobial resistant bacteria isolated from animal species, which leads to difficulty in estimating the scale of the problem and its potential impact on animal and human health. Thus, the objective of this PhD project was to study the molecular epidemiology of ESBL/AmpC producing *E. coli* (ESBL/AmpC-E) and MRSA from companion animals in New Zealand.

The thesis starts with an overview of the literature on ESBL and AmpC producing bacteria and MRSA, their resistance mechanisms and the clinical significance of the infections in humans and animals (Chapter 1). This is followed by five observational epidemiological studies of ESBL and AmpC-producing bacteria and MRSA in companion animals (Chapters 2 – 6). The thesis was written in the form of individual publications; thus, some repetitions were inevitable, especially in the introduction sections of the different chapters. A pilot study aimed at selecting the optimal method for the isolation of ESBL/AmpC-E is reported in Appendix 1 and the raw data collected in this project is in the attached CD.

The first observational study (Chapter 2) was performed in co-operation with New Zealand veterinary diagnostic laboratories, with the aims of (1) establishing whether ESBL/AmpC-E are causing clinical infections in companion animals, and (2) genetically characterise the strains. This was

followed with an epidemiological study (

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Name of Candidate: Ali Karkaba

Name/Title of Principal Supervisor: Alex Grinberg

Name of Published Research Output and full reference:

Karkaba, A. Grinberg, J. Benschop, E. Pleydell (2016). Characterisation of extended spectrum β-lactamase and AmpC β-lactamase-producing Enterobacteriaceae isolated from companion animals in New Zealand. New Zealand Veterinary Journal

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Chapter 3) performed in co-operation with veterinary practices in Auckland, to estimate the faecal carriage of these bacteria by cats and dogs (

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Chapter 3). Chapter 4 assessed the risk factors associated with the faecal carriage by cats and dogs. Phenotypic and molecular analysis of the strains

reported in Chapter 2 and

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Chapter 3 inform Chapter 5, which assesses the clonal relatedness of ESBL/AmpC-E across faecal carriage and disease.

The last study (Chapter 6) characterised MRSA identified from clinical infections in animals in New Zealand, and estimated the prevalence of MRSA carriage in dogs and cats in Auckland.

All the studies in this thesis were conducted between June 2012 and June 2013. Chapter 2 and Chapter 6 have been published in condensed form in refereed journals and were also presented at conferences.

Declaration

This thesis contains no material that has been accepted for another degree or diploma at Massey University or any other institution. To the best of my knowledge, no material previously published or written by another person has been used, except where due acknowledgment has been made in text.

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Gratitude perpetuates blessings. Words are not enough to show my gratitude for a lot of people that helped me during this journey. I would like to thank my main supervisor Alex Grinberg for his mentoring, patience, encouragement, endless hours of meetings, supervision and support. Besides my main supervisor, I would like to thank the PhD co-supervisors, Eve Pleydell, that had a major role at the beginning of this PhD studies and left for another job; Kate Hills' role at the beginning of the studies that also left; lastly but not least, Jackie Benschop that took the burden of the co-supervision, and the important role in mentoring, guidance and support during the PhD studies.

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Heartfelt thanks to my family that without them I wouldn't be here.

List of contents

List of Tables

List of Figures

List of Abbreviations

E. coli	*Escherichia coli*
ESBL	Extended spectrum β-lactamase
AmpC	AmpC β-lactamase
ESBL-E	Extended spectrum β-lactamase producing *E. coli*
AmpC-E	AmpC β-lactamase producing *E. coli*
ESBL/AmpC-E	ESBL-E and/or AmpC-E
MLST	Multilocus sequence typing
ST	Sequence type
MALDI-TOF	Matrix assisted laser desorption/ionization time of flight
AMR	Antimicrobial resistant
MDR	Multi-drug resistant
ExPEC	Extra-intestinal pathogenic *E. coli*
HGT	Horizontal gene transfer
S. aureus	*Staphylococcus aureus*
MRSA	Methicillin resistant *Staphylococcus aureus*
SCC*mec*	Staphylococcal cassette chromosome

List of publications

Chapter 2: Karkaba, A. Grinberg, K. E. Hill, J. Benschop (2017). Characterisation of methicillin resistant *Staphylococcus aureus* clinical isolates from animals in New Zealand, 2012–2013, and subclinical colonisation in dogs and cats in Auckland. *New Zealand Veterinary Journal.*

Chapter 6: Karkaba, A. Grinberg, J. Benschop, E. Pleydell (2017). Characterisation of extended spectrum β-lactamase and AmpC β-lactamase-producing Enterobacteriaceae isolated from companion animals in New Zealand. *New Zealand Veterinary Journal*.

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Oral conference presentations:

A. Karkaba, K. Hill, J. Benschop, A. Grinberg and E. Pleydell (2013). Investigating the strains of multidrug resistant Enterobacteriaceae and *Staphylococcus aureus* that are causing clinical infections in companion animals in New Zealand. *Proceedings of 38th Annual WSAVA Congress.*

Karkaba, A., Hill, K., Benschop, J., Grinberg, A. and Pleydell, E. (2013). Comparison between five methods for isolation of multi drug-resistant *Escherichia coli* from faeces of hospitalized and non-hospitalized cats and dogs *Queenstown Molecular Biology Week and Webster Centre for Infectious Diseases Symposium: Of Microbes and Men – Translational Medical Microbiology in the 21st Century*. 29 – 30 August, 2013 Rydges Hotel, Queenstown, New Zealand

A. Karkaba, K. Hill, J. Benschop, A. Grinberg and E. Pleydell (2012). Investigating the strains of multidrug resistant Enterobacteriaceae causing clinical infections in companion animals in New Zealand. *Proceedings of First IDReC Science Symposium.*