Identification and Molecular Characterization of Serotypes and Virulence Factors of *Klebsiella pneumoniae* from Mastitic Dairy Cattle of Batangas, Philippines

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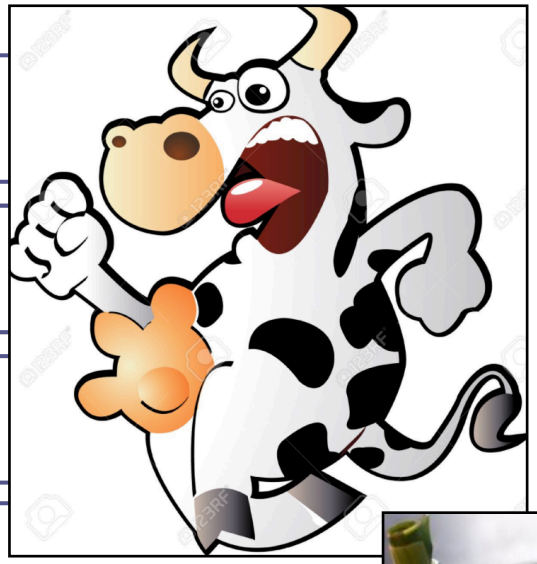


- Bovine mastitis
  - inflammation of the mammary gland caused by several bacteria (Zadoks *et al.*, 2011) but is also a response to intramammary mycoplasmal, fungal, or algal infections.
  - most costly disease in dairy cattle. Decreased milk production accounts for ~70% of the total cost of mastitis (Zhao & Lacasse, 2008).

- Bovine mastitis

Classifications

1. Subclinical - mild non-visible inflammation of mammary gland w/ normal milk and quarter
2. Clinical - visible abnormal, clotty or flaky appearance of the milk even w/ normal udder

Types

1. Contagious - spread from cow to cow, via milkers' hands, milking machine, and flies
2. Environmental - fecal origin or from the surroundings such as the beddings, feed and soil

Klebsiella pneumoniae

Fig 1. *Klebsiella pneumoniae* in McConkey agar

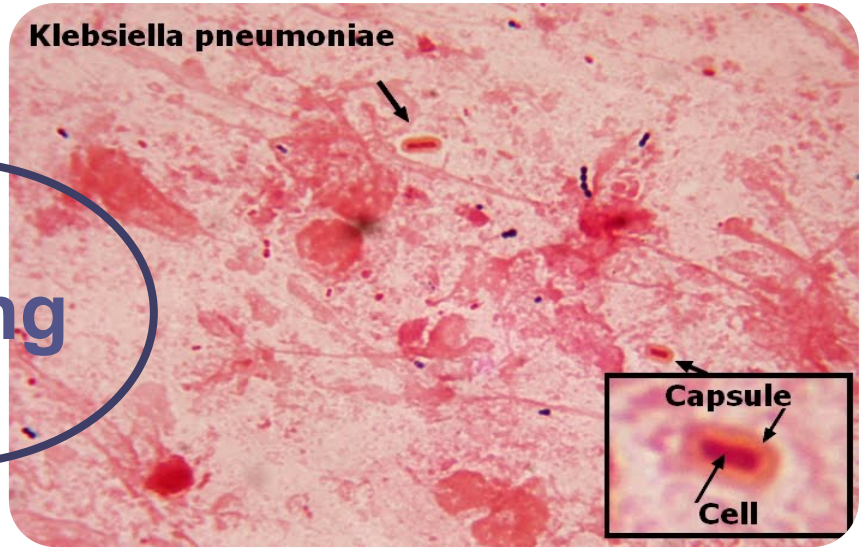


Fig 2. Light microscopy of *Klebsiella pneumoniae*

Typing

Pathogenesis

1. Capsular antigens
2. Adhesins
3. Siderophore
4. Lipopolysaccharide

Klebsiella pneumoniae

Humans

1. Bacteremia
2. Respiratory infection
3. Urinary infection
4. Pyogenic liver abscess
5. Meningitis
6. Endophthalmitis

Animals

1. Similar in humans - dogs, monkeys, guinea pigs, muskrats, birds and fox
2. Mastitis - cattle
3. Metritis - mares

Klebsiella pneumoniae

Table 1. List of lesions caused by common *K. pneumoniae* serotypes.

#	Serotype	Symptom / Clinical Sign	Reference
1	K1	Invasive pyogenic liver abscess, septicemia, pneumonia, endopthalmitis, metritis (mares)	Brisse *et al.*, 2009 & Turton *et al.*, 2010
2	K2	pneumonia, metritis (mares), resistance to neutrophil phagocytosis	Brisse *et al.*, 2009
3	K5	pneumonia, metritis (mares), resistance to neutrophil phagocytosis	Brisse *et al.*, 2009
4	K20	Invasive pyogenic liver abscess, septicemia, pneumonia	Turton *et al.*, 2010
5	K54	Invasive pyogenic liver abscess, septicemia, pneumonia	Turton *et al.*, 2010
6	K57	Invasive pyogenic liver abscess, septicemia, pneumonia	Turton *et al.*, 2008

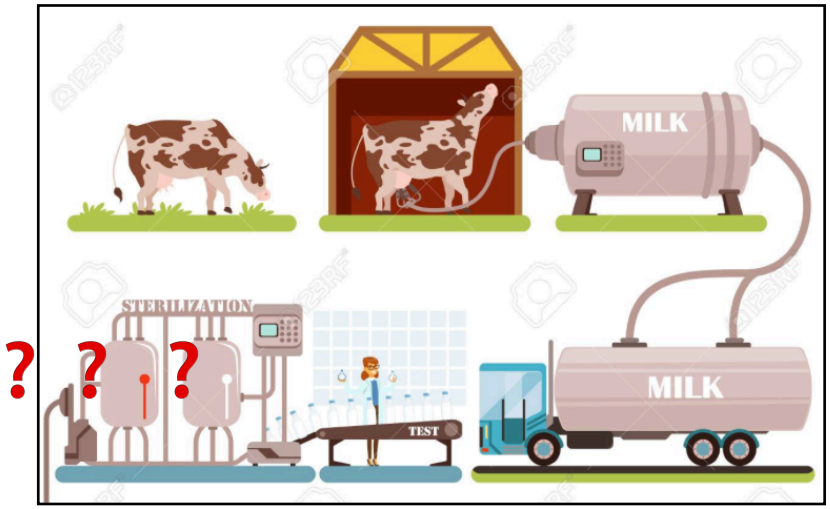
Klebsiella pneumoniae

Table 2. List of virulence factors associated with *K. pneumoniae* plasmid-borne virulence genes.

#	Virulent Gene	Virulence factor	Reference
1	*mag*A	hypermucoviscosity, serum and phagocytosis resistance	Brisse *et al.*, 2009
2	*wzy*	hypermucoviscosity	Chuang *et al.*, 2006
3	K5*wzx*	hypermucoviscosity, serum and phagocytosis resistance	Chuang *et al.*, 2006
4	*rmp*A	hypermucoviscosity	Brisse *et al.*, 2009
5	*wca*G	phagocytosis resistance	Brisse *et al.*, 2009

Research problem and its significance

➢ Why *Klebsiella pneumoniae* ???



Hypothesis:

Klebsiella pneumoniae isolated in the bovine mastitic milk are pathogenic.

Objectives of the study

The study aims to establish the prevalence, to determine the molecular serotypes and to understand the virulence factors of *Klebsiella pneumoniae* isolates from bovine mastitis milk.

Outcome

- To help prevent and control the pathogenicity of *Klebsiella pneumoniae* which is essential in alleviating mastitis;
- To help diminish or avoid transfer of such virulence factors to other pathogens that could infect humans.

Time and place of the study

- Time: June 2016 to June 2018

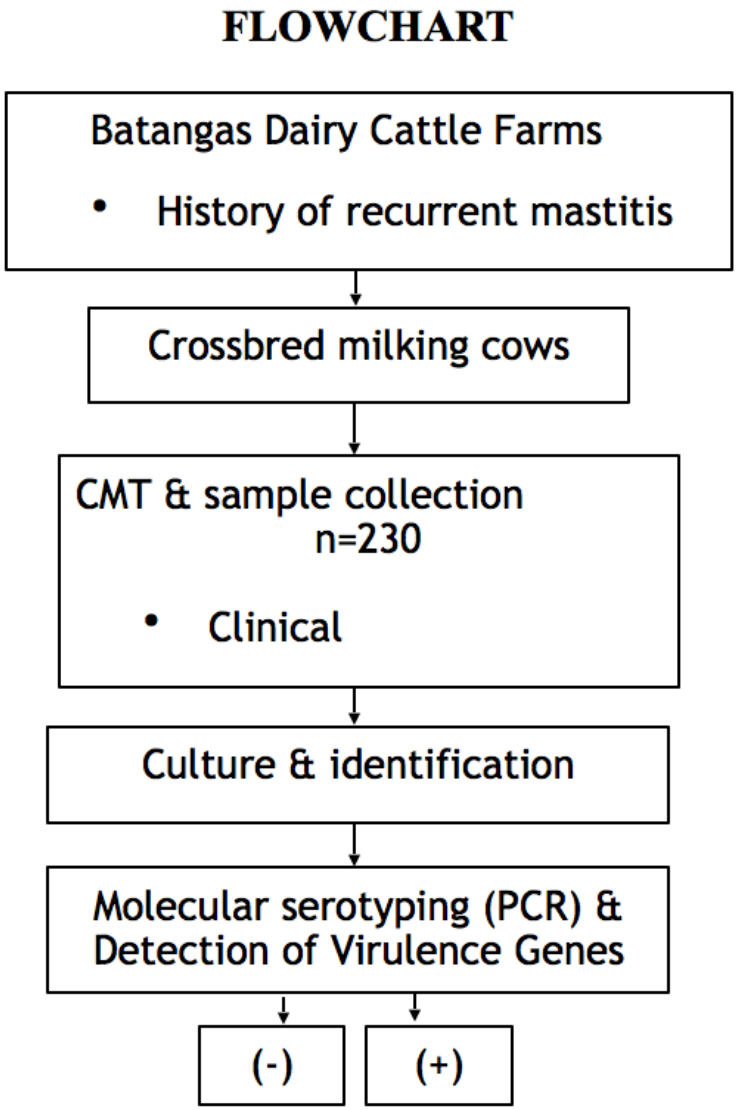
- Location:

➢DVPS, CVM, UPLB, Laguna, &

➢Dairy cattle farms in Batangas

Fig 3. Philippine map showing northeastern Batangas as a dairy cattle zone

Materials & Methods



Materials & Methods

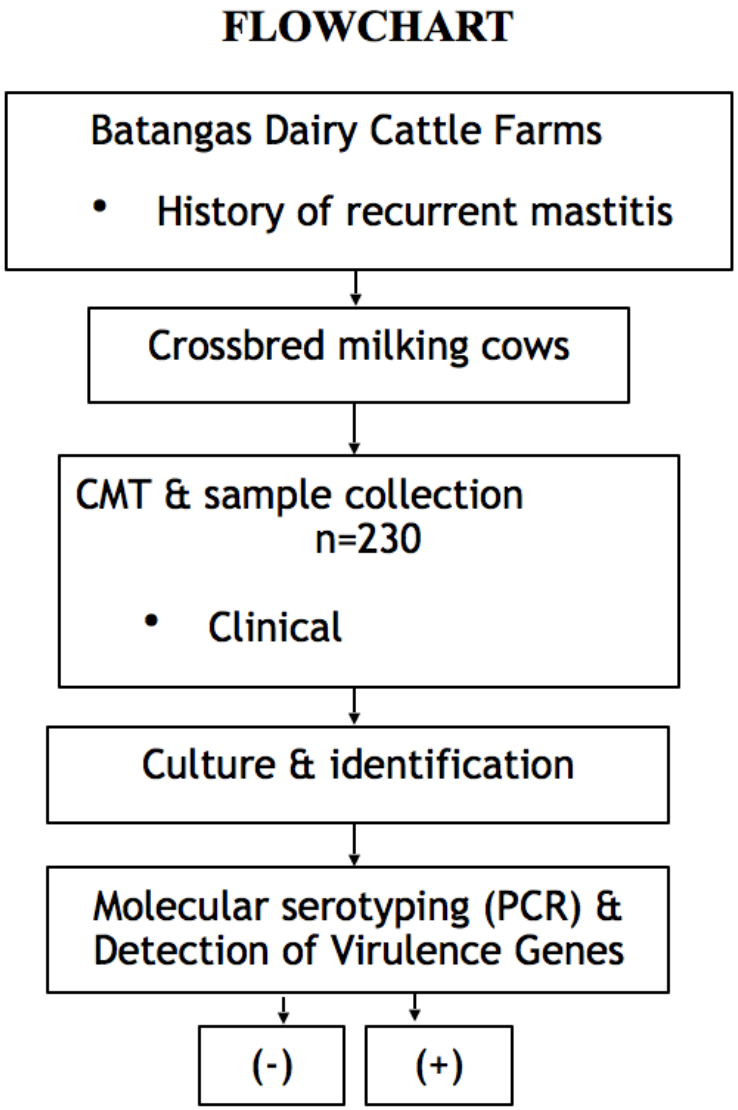
- Clinical mastitis screening

Fig 4. CMT kit

Table 3. California Mastitis Test (CMT) scores (Ruegg, P.L., 2005)

CMT score	Somatic Cell Range	Interpretation
N (negative)	0-200,000	Healthy quarter
T (trace)	200,000-400,000	Subclinical mastitis
1	400,000-1,200,000	Subclinical mastitis
2	1,200,000-5,000,000	Serious mastitis infection
3	Over 5,000,000	Serious mastitis infection

Materials & Methods



Materials & Methods

- Beta actin gene and molecular detection

Table 4. List of primers used in this study for detection of integron and gene cassette.

#	Gene	Size (bp)	Primers	Sequence (5'-3')	Reference
1	β-actin	210	BAG-F	CGCACCACCGGCATCGTGAT	Tajima *et al*., 1998
2			BAG-R	CGCACCACCGGCATCGTGAT	
3	*K. pneumoniae*	130	*K. pneumoniae* Pf	ATTTGAAGAGGTTGCAAACGAT	Liu *et al*., 2008
4	16S– 23S ITS		*K. pneumoniae* Pr1	ATTTGAAGAGGTTGCAAACGAT	

Materials & Methods

- Molecular serotyping

Table 5. List of primers used in this study for detection of serotypes and virulence genes.

#	Gene	Size (bp)	Serotype	Primers	Sequence (5'-3')	Reference
1	*magA*	1283	K1	*magAF1*	GGTGCTCTTTACATCATTGC	Turton *et al.*, 2008
2				*magAR1*	GCAATGGCCATTTGCGTTAG	
3	*wzy*	641	K2	*wzy*-F1	GACCCGATATTCATACTTGACAGAG	Turton *et al.*, 2008
4				*wzy*-R1	CCTGAAGTAAAATCGTAAATAGATGGC	
5	K5*wzx*	280	K5	K5wzxF360	TGGTAGTGATGCTCGCGA	Turton *et al.*, 2008
6				K5wzxR639	CCTGAACCCACCCCAATC	
7	*rmpA*	516	Non-K1/ K2	*rmpAF*	ACTGGGCTACCTCTGCTTCA	Yeh, *et al.*, 2007
8				*rmpAR*	CTTGCATGAGCCATCTTTCA	
9	K20	741	K20	*wzyK20F*	CGGTGCTACAGTGCATCATT	Fang, *et al.*, 2007
10				*wzyK20R*	GTTATACGATGCTCAGTCGC	
11	K54	881	K54	*wzxK54F*	CATTAGCTCAGTGGTTGGCT	Fang, *et al.*, 2007
12				*wzxK54R*	GCTTGACAAACACCATAGCAG	
13	K57	1037	K57	*wzyK57F*	CTCAGGGCTAGAAGTGTCAT	Fang, *et al.*, 2007
14				*wzyK57R*	CACTAACCCAGAAAGTCGAG	
15	*wcaG*	169	*wcaG*	*wcaGF*	GGTTGGKTCAGCAATCGTA	Turton *et al.*, 2010
16				*wcaGR*	ACTATTCCGCCAACTTTTGC	

Results & Discussion

BATANGAS

12 dairy cattle farms

CMT
624 milking cows
2,406 teats

Subclinical mastitis rate = 9%
Clinical mastitis rate = 19%

Individual quarter milk samples (n=230)

11 isolates from McConkey-inositol-potassium tellurite agar = 4.78% prevalence rate

colony morphology, biochemical testing & molecular detection

6 isolates = 2.6% confirmed prevalence rate

A273 D02 D74B F6505 G26 G40

Results & Discussion

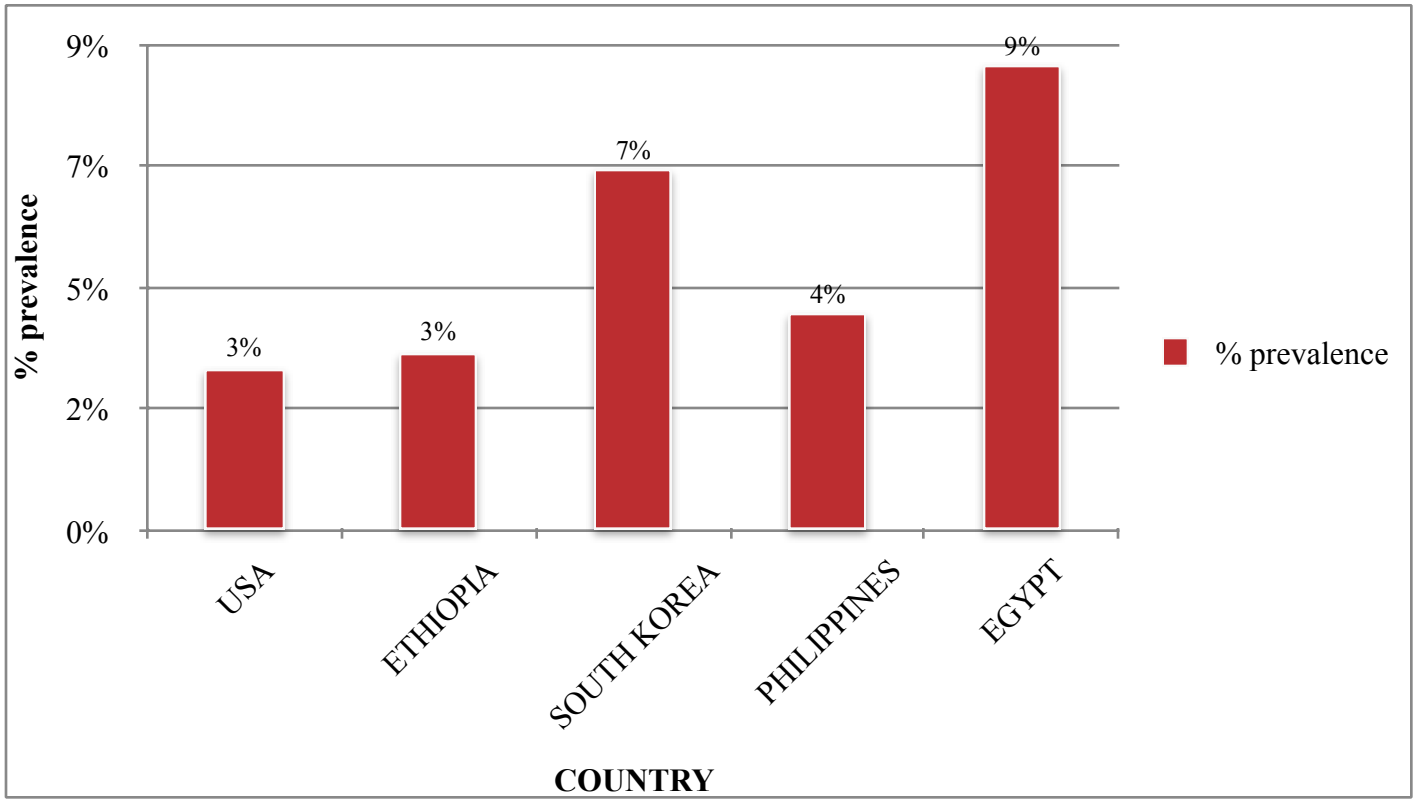


Fig 5. Prevalance rates of *K. pneumoniae* bovine mastitis from previous studies worldwide (2004 - 2014).

Results & Discussion

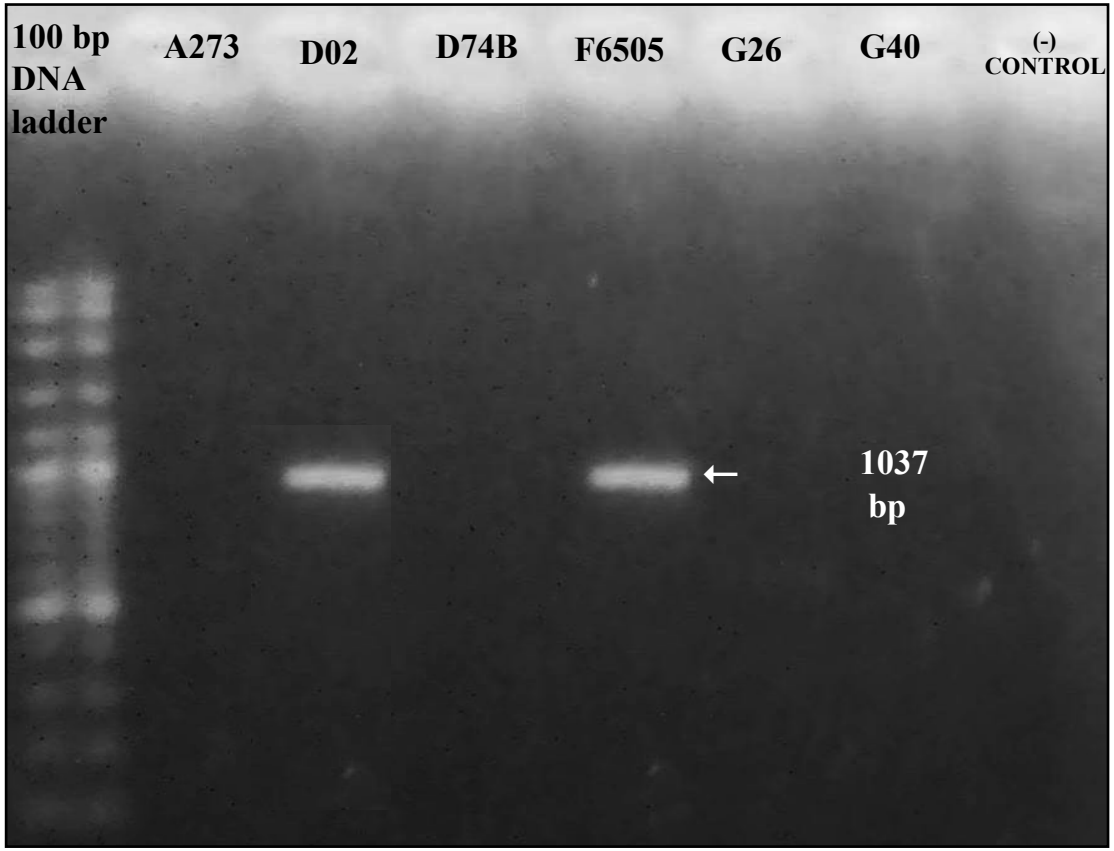


Figure 5. Representative picture of PCR amplicon of K57 (1037 bp) present in D02 and F6505 isolates. Lane 1: 100 bp DNA ladder, Lane 8: negative control, Lane 2, 4, 6-7: negative isolates, Lane 3 & 5: positive isolates.

Results & Discussion

Table 3. List of serotypes & virulence genes found in *Klebsiella pneumoniae* isolates (n=6).

#	Serotype	Virulence genes	Number (%)
1	K1	*mag*A	0
2	K2	*wzy*	0
3	K5	K5*wzx*	0
4	Non-K1/K2	*rmp*A	0
5	K20	N.A.	0
6	K54	N.A.	0
7	K57	N.A.	2 (33%)
8	N.A.	*wca*G	0

Results & Discussion

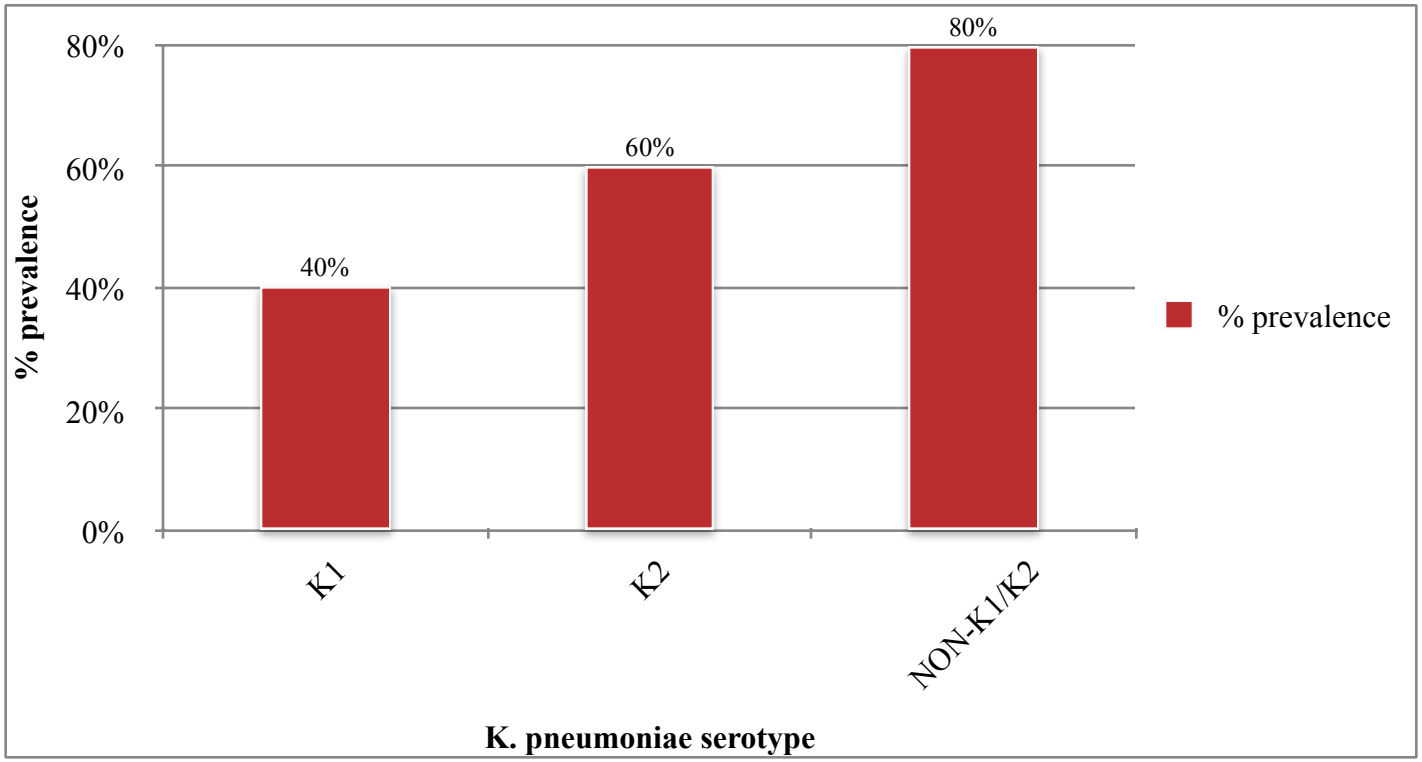


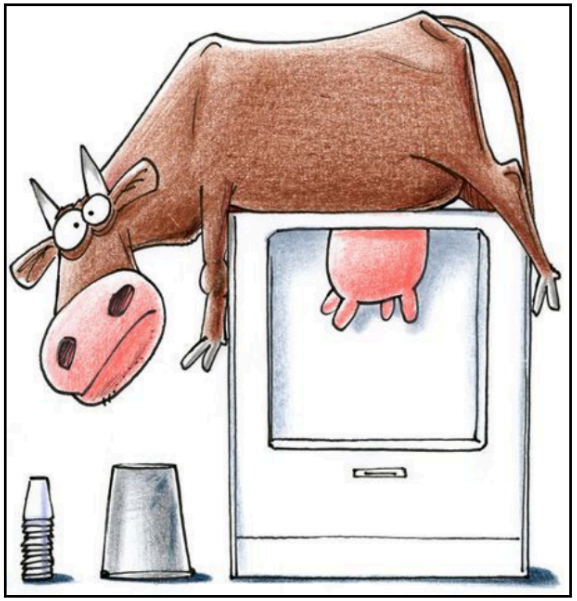
Fig 5. Prevalance rates of common serotypes of *K. pneumoniae* bovine mastitis from Osman, et al. 2014.

Summary & Conclusion

- Since there were no virulence genes detected even on the K57 isolate, it could be noted that the infections brought about by these isolates are not that severe.

- *Klebsiella pneumoniae* isolated from mastitic milk samples of dairy cattle…

1st in the Philippines to account for molecular serotypes and virulence factors



Way forward…

- Continue with the detection of molecular serotypes of remaining four isolates
- Obtain samples from more farms
- Continue obtaining samples from previously enrolled farms as part of the surveillance program
- Continuously employ higher standards on the operations of dairy cattle farms and processing plants to have a higher quality milk

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Acknowledgment

Thank you!!!

Maraming Salamat po!!!

Khop Khun Kha!!!