UNIVERSITI PUTRA MALAYSIA

OCCURRENCE OF MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII AND ESCHERICHIA COLI IN VETERINARY HEALTH CARE FACILITIES

JOANNA NG SZE YI

OCCURRENCE OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* AND *ESCHERICHIA COLI* IN VETERINARY HEALTH CARE FACILITIES

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It is hereby certified that we have read this project paper entitled "Occurrence of Multidrug-resistant *Acinetobacter baumannii* and *Escherichia coli* in Veterinary Health Care Facilities", by Joanna Ng Sze Yi and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project.

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To Mom, Dad, Ah Mah and in loving memory of Ah Gong…

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KEHADIRAN *ACINETOBACTER BAUMANNII* DAN *ESCHERICHIA COLI* RINTANG-MULTIDRUG PADA KEMUDAHAN KESIHATAN VETERINAR

Oleh

Joanna Ng Sze Yi

2015

Penyelia: Prof. Dr. Saleha Abdul Aziz

Organisma rintang-multidrug (MDROs) seperti rintang-multidrug (MDR) *Acinetobacter baumannii* dan *Escherichia coli* adalah patogen penting yang berkaitan dengan jangkitan nosokomial di persekitaran kemudahan kesihatan manusia dan haiwan. Permukaan objek di kemudahan kesihatan boleh dijadikan sebagai sumber jangkitan. Walaubagaimanapun, kajian mengenai prevalens patogen di persekitaran kemudahan kesihatan veterinar adalah kurang di negara ini. Oleh itu, objektif kajian ini adalah untuk menentukan kehadiran *A. baumannii* dan *E. coli* dan kehadiran MDR *A. baumannii* dan *E. coli* yang diasingkan daripada permukaan objek di kemudahan kesihatan veterinar di Lembah Klang, Malaysia. Dalam kajian ini, sampel swab diambil daripada 65 permukaan termasuk pemegang pintu, meja pemeriksaan, pakaian makmal, stetoskop dan alat timbang di empat kemudahan kesihatan veterinar. Sampel swab dikultur dan dikenalpasti dan semua isolat telah diuji kerintangan antibiotik. Hasil kajian ini menunjukkan bahawa kahadiran *A. baumannii* adalah 9.23% dan lima daripada enam isolat *A. baumannii* (83.33%) telah dikelaskan sebagai MDR.

Walaubagaimanapun, *E. coli* tidak diasingkan. Kesimpulannya, permukaan objek boleh menjadi sumber MDR *A. baumannii* di kemudahan kesihatan veterinar.

Kata kunci: *Acinetobacter baumannii, Escherichia coli,* jangkitan nosokomial, ujian kerintangan antibiotik.

ABSTRACT

OCCURRENCE OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* AND *ESCHERICHIA COLI* IN VETERINARY HEALTH CARE FACILITIES

By

Joanna Ng Sze Yi

2015

Supervisor: Prof. Dr. Saleha Abdul Aziz

Multidrug-resistant organisms (MDROs) such as multidrug-resistant (MDR) *Acinetobacter baumannii* and *Escherichia coli* are important pathogens associated with nosocomial infections in both human and animal health care settings. Surfaces of inanimate objects in health care facilities can serve as important sources of infection. However, studies on prevalence of these pathogens in veterinary settings are lacking in the country. Therefore, the objectives of this study were to determine the occurrence of *A. baumannii* and *E. coli* and the occurrence of MDR isolates on surfaces of inanimate objects from veterinary health care facilities in Klang Valley, Malaysia. In this study, swab samples were taken from 65 surfaces of inanimate objects which included door knobs, examination tables, labcoats, stethoscopes and weighing scales in veterinary health care facilities. The swab samples were cultured and all isolates were subjected to antibiotic susceptibility testing. Results of this study revealed the occurrence of *A. baumannii* was 9.23% and five out of six *A. baumannii* isolates (83.33%) were classified as MDR. However, no *E. coli* was isolated. In conclusion, surfaces of inanimate objects can be a source of MDR *A. baumannii* in veterinary health care facilities.

Keywords: *Acinetobacter baumannii*, *Escherichia coli*, nosocomial infection, antibiotic sensitivity test.

1.0 Introduction

Hospital-associated infections (HAI) or also known as nosocomial infections have become increasingly important in both human and animal health care settings. However the role of veterinary hospital environment in HAI is largely unknown. Apart from complicating treatments of hospitalized patients, HAI also impose risk to health care personnel and pet owners (Stull & Weese, 2015).

Multidrug-resistant organisms (MDRO) are often involved in HAI. As defined by its name, MDROs limits options treatment and may worsen prognosis of patients. However, MDROs are not inherently more virulent than microbial susceptible organisms (Stull & Weese, 2015). MDROs that are of concern in small animal clinics are namely *Acinetobacter baumannii*, *Escherichia coli*, *Enterococcus* spp, *Salmonella* spp, *Staphylococcus* spp, and *Pseudomonas* spp (Stull & Weese, 2015).

Over the last decade, *Acinetobacter baumannii* have emerged as a significant opportunistic nosocomial pathogen and have caused several hospital and veterinary outbreaks (Consales *et al.,* 2011; Brito *et al.,* 2005). This gram negative, non-fermenting bacillus survives for a prolonged period under a wide range of environmental condition (Maragakis & Perl, 2008). Therefore, its ability to survive on dry surfaces has made health care facilities to be an important source of infection for patients and health care personnel (Jawad *et al.,* 1998). This pathogen can cause pneumonia, bloodstream infection, urinary tract infection (UTI), skin and soft tissue infection in both humans and animals (Francey *et al.,* 2000; Peleg *et al.,* 2008). *Acinetobacter baumannii* had emerged

as one of the most troublesome pathogen due to its ability in acquiring resistance determinants which results in treatment failure. Most importantly, strains that are resistant to all known antibiotic were reported to cause an alarming threat to the antibiotic era (Peleg *et al.,* 2008).

Escherichia coli are a large and diverse group of gram negative bacilli. They can be found in the ubiquitously in the environment, and are important normal flora of the gut of warm blooded animals (CDC, 2014). However, some strains of *E. coli* are pathogenic and can cause severe illness that can lead to death.

Johnson *et al.* (2001) proposed that dog faeces can be a possible reservoir of *E. coli strains* that causing extraintestinal infection in humans, since there is clonal commonality between canine faecal *E. coli* and human clinical *E. coli* isolates.

Apart from all the above, Malaysia is lack of information regarding the prevalence of these two MDR bacteria especially in veterinary health care settings. Therefore, the hypothesis of this study is high prevalence of MDR *A. baumannii* and *E. coli*. The objectives of this study are to determine the prevalence of *A. baumannii* and *E.coli* on surfaces of inanimate objects in veterinary facilities and to determine the multidrug-resistance of the isolates.

2.0 Literature review

2.1 *Acinetobacter baumannii*

2.1.1 General description of *A. baumannii*

Acinetobacter baumannii is a gram negative bacteria which belongs to the family of *Moraxellaceae.* The name "Acinetobacter" originates from a Greek word "akinetos" which means unable to move (Doughari *et al.,* 2011). It is strictly aerobic, non-motile, catalase-positive, indole-negative, oxidase-negative, non-fermentative encapsulated coccobacilli (Singh *et al.,* 2013).

2.1.2 Clinical manifestation of *A. baumannii*

According to Centers for Disease Control and Prevention (CDC) there are many species of *Acinetobacter spp.* and all can cause disease. However, *Acinetobacter baumannii* accounts about 80% of reported infections in humans (CDC, 2010). Peleg *et al.* (2008) stated that *A. baumannii* can cause pneumonia, bloodstream infection and occasionally skin infection, urinary tract infection (UTI), meningitis and soft tissue infection in humans. In animals, *A baumannii* were isolated from patients suffering from UTI, pyothorax, upper airway obstruction, bloodstream infection and wound infection (Francey *et al.,* 2000).

In a six years study following 505 patients with nosocomial bacteraemia, Jerassy *et al.* (2006) concluded that in-hospital mortality in patients with *A. baumannii* bacteraemia (57%) was significantly higher than bacteraemia cause by other gram negative organism (31-43%). Another study also shows that there is an increase in mortality with multidrug-resistant *A. baumannii* colonization or

infection compared to that with multidrug-resistant *Pseudomonas aeruginosa* colonization or infection (Gkrania-Klotsas & Hershow, 2006).

2.1.3 Epidemiology of *A. baumannii*

Acinetobacter baumannii can be found ubiquitously especially in soil, water, animals and humans (Baumann, 1968; Fournier & Richet, 2006). A study in Reunion Island found that the prevalence of *A. baumannii* in mouth, rectum and wound was 6.5% (Belmonte et al., 2012). *Acinetobacter baumannii* is an opportunistic pathogen. Therefore, infections caused by this pathogen are usually found in patients that are ill or immunosuppressed. Prata-Rocha *et al.* (2012) described the factors that are associated with mortality in patients with MDR *A. baumannii* infection which includes: Patients age > 60 years, patients suffering from pneumonia, diabetes mellitus, renal disease, use of more than two invasive procedures, use of more than 2 medical devices and inappropriate antimicrobial therapy.

The ability of an organism to survive on dry surface is important to determine if surfaces of inanimate objects can be a source of infection especially in health care settings. A study conducted in 1998 proved that the survival times of sporadic strains of *A. baumannii* is 27.2 days and outbreak strains survives for 26.5 days on dry surfaces. However, the survival time for both strains showed that they were not statistically different (Jawad *et al.*, 1998).

In Malaysia, MDR *A. baumannii* is a common isolates from intensive care unit of human hospital (Kong, 2011; Lean *et al.*, 2014). However, such studies were not done in veterinary patients and environment of veterinary facilities of Malaysia.

2.1.4 Antimicrobial resistance of *A. baumannii*

Bacteria isolates that acquired non-susceptibility to at least one agent in three or more antimicrobial categories are classified as MDR (Magiorakos *et al.*, 2012). Nosocomial *A. baumannii* isolates are mostly multidrug resistant and antimicrobial susceptibility testing showed that outbreak strains were significantly more resistant to various broad-spectrum antimicrobial agents than sporadic strains (Jawad *et al.,* 1998). Apart from that, extensive drug resistant (XDR) *A. baumannii* which are resistant to all but one or two classes of antibiotics and even pandrug resistant (PDR) isolates that are resistant to all classes of antibiotics are emerging at an alarming rate. A study using strains isolated from a main tertiary hospital in Terengganu showed that out of the 54 isolates, 39 (72.2%) were multidrug resistant (MDR) and resistant to carbapenems whereas 14 (25.9%) were categorized as extensive drug resistant (XDR) with additional resistance to polymyxin B, the drug of "last resort."(Lean *et al.*, 2014)

Another investigation done by Kuo *et al.* (2012) by using date from Taiwan Surveillance of Antimicrobial Resistance (TSAR) revealed that XDR *A. baumannii* have increased from 1.3% in 2002 to 41.0% in 2010. As for PDR strains, rapid emergence (from 0% before 1998 to 6.5% in 2000) of PDR *A. baumannii* was noted in a university hospital in Taiwan. It was believed that increasing use of carbapenems and ciprofloxacin possessed selective pressure as well as clonal dissemination might have contributed to this phenomenon (Hseuh, 2002).

2.2 *Escherichia coli*

2.2.1 General description of *E. coli*

Escherichia coli is a gram negative, facultative anaerobic, non-spore-forming, motile rod which belongs to the family *Enterobacteriaceae*. The genus was named after Theodor Escherich, the person who first isolated *E. coli* in 1884 (Schaechter & Lederberg, 2004).

2.2.2 Clinical Manifestation of *E. coli*

Escherichia coli are opportunistic pathogens that can cause diarrhoea, urinary tract infection (UTI), respiratory illness, bloodstream infection, surgical site infection, meningitis and other illnesses in both humans and animals (CDC, 2014; Sanchez *et al*, 2002; Drazenovich, 2004). However, some *E. coli* are pathogenic and they are classified into pathotypes based upon the diseases that they cause and the virulence factors that they possess (Nataro *et al.*, 2004). Pathogenic *E. coli* can be classified as either extra-intestinal pathogenic E. coli (ExPEC) or intestinal pathogenic *E. coli* (InPEC) based upon the anatomical site in which diseases occur.

ExPEC strains usually cause infections outside of the intestinal tract such as urinary tract infections, neonatal meningitis and septicaemia. *E. coli* have been the most common etiological agent that cause UTI in humans, cats and dogs (Seguin *et al.*, 2003; Litster *et al.*, 2009; Farajnia *et al.*, 2009). However, they have the ability to colonize the intestinal tract without causing disease. In contrast, intestinal colonization by InPEC strains can cause different types of

gastroenteritis with different infection mechanisms and symptoms. InPEC can be divided into six pathogenic groups: enterohaemorrhagic (EHEC); enteropathogenic (EPEC); enteroaggregative (EAEC); enterotoxigenic (ETEC); enteroinvasive (EIEC) and diffusely adherent (DAEC) E. coli (Nataro, 2004).

2.2.3 Epidemiology of *E. coli*

Escherichia coli can be found ubiquitously in the environment and they are normal flora of the gut in warm blooded animals. Most *E coli* are harmless (non-pathogenic) and do not cause disease in healthy individual (CDC, 2014). However, they can be opportunistic pathogen that can cause severe diseases when there is a break in the immune system. For example, prolonged usage of urinary catheter would risk the patients in acquiring catheter-associated urinary tract infections due to *E. coli* (Jacobsen *et al.*, 2008).

The predisposing factor in acquiring *E. coli* urinary tract infection in human study as described by Marschall *et al.* (2012) are urinary stasis, surgical disruption of urinary tissues and benign prostatic hyperplasia. In dogs, the urinary tract is the most common infection site and urinary catheterization, bladder stasis, or both were common among dogs (24%). From the same study, 97% of dogs that had extra-intestinal *E. coli* infection had an underlying disease, 87% received prior antimicrobial treatment, 82% were hospitalized for 3 days or more, and 57% had a surgical intervention (Gibson *et al.*, 2008).

2.2.4 Antimicrobial resistance of *E.coli*

Most studies show that after the introduction of an antibiotic not only the level of resistance of pathogenic bacteria, but also of commensal bacteria increases. This is a concern as commensal bacteria can serve as a reservoir of resistance genes for pathogenic bacteria. Therefore, apart from monitoring the prevalence of resistance in indicator bacteria such as faecal *E. coli* and enterococci in humans and animals it also allow us to detect transfer of resistant bacteria or resistance genes from animals to humans and vice versa (Bogaard & Stobbeingringh, 2000).

Multidrug-resistant *E.coli* are commonly found in hospital settings and are increasingly being isolated in the community (Ibrahim *et al.,* 2012). Antibiotic resistance of most MDROs are often seen in commonly used antibiotics. In a retrospective study conducted by Kibret and Abera (2011) by using clinical source of *E. coli* in northeast Ethiopia, high resistance rates to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%) were documented. However, there were significantly high degree of sensitivity rates towards nitrofurantoin (96.4%), norflaxocin (90.6%), gentamicin (79.6%) and ciprofloxacin. In dogs and cats, resistance was observed towards streptomycin (96.4%), neomycin (85.1%), amoxicillin (70.2%), and gentamicin (68.1%) (Magdalena *et al.*, 2015). Furthermore, the percentage of MDR isolates had been increasing at an alarming rate in clinical isolates of cats and dogs from 50.0% in 2007-2008 to 89.9% in 2013 (Magdalena et al., 2015).

3.0 Materials and Methods

3.1 Specimen Collection

Swabs of 65 surfaces of inanimate objects in four veterinary health care facilities of Klang Valley were taken. Types of inanimate objects and sampling site of each object are summarized in Table 1.

Table 1: List of inanimate objects and the area where swabs samples were taken

Types of inanimate objects	Number of objects sampled	Sampling site
Door handles	18	Whole surface
Examination tables	18	$100cm^3$ at the center of the table top
Labcoats	9	3 cm wide at the posterior end of sleeves and $100cm^3$ at the abdomen above the level of navel.
Stethoscopes	9	Bell and diaphragm
Weighing scale	9	100cm3 at the center of the weighing platform
Animal cage	2	100cm3 at the center of cage floor

By using sterile swabs pre-moistened with phosphate buffered saline (PBS), two swabs samples were taken simultaneously on each surface (Figure 1).

Figure 1: Swabs samples taken from a door handle using sterile swabs pre-moistened with phosphate buffered saline (PBS).

3.2 Bacterial Isolation and Identification

Samples were kept in an ice box and transported immediately to laboratory for isolation and identification. Samples were cultured on MacConkey agar (for isolation of *Acinetobacter baumannii*) and Chromocult® Coliform Agar (CCA) (for isolation of *Escherichia coli)* and incubated overnight at 37ºC. All gram negative bacteria that grew on MacConkey and CCA agar were subcultured after gram staining for another 24 hours in 37ºC. For identification of *E. coli*, a drop of KOVACS' reagent was placed directly on dark purple colonies on CCA. Colonies of *E. coli* would turn the reagent into cherry red within seconds. All other gram negative colonies were subjected to biochemical tests as described by Jang *et al.* (2008) such as triple sugar iron agar (TSI), sulfa-indole motility test (SIM), citrate, and urease test.

Suspected *A. baumannii* which match all biochemical results were further grown at 41 ºC and 44 ºC. The identified *Acinetobacter* spp. were further confirmed up to genus level by using RapID™ NF Plus identification system. RapID™ NF Plus is an identification system based on enzyme technology. It consists of a clear plastic tray which contains 10 reagent impregnated wells. A suspension of test organism in RapID Innoculation Fluid was used as the inoculum which rehydrate and initiated test reactions. Other gram negative bacteria such as *Pseudomonas aeruginosa, Alcaligenes feacalis,* and *Moraxella* sp. were also tested by using RapID™ NF Plus.

3.3 Antibiotic Susceptibility Test

The antibiotic susceptibility test using Kirby Bauer disk diffusion method were performed in all isolates. The antibiotic tested, concentration of the antibiotic disk used and the antibiotic susceptibility interpretative criteria are summarized in Table 2.

Table 2: Antibiotic Susceptibility Interpretative Criteria as described by CLSI VET01-S2 guideline (2013)

Antimicrobial agent	Disk content	Zone diameter (mm)		
		S	I	R
Amoxicillin/clavulanic acid*	30µg	≥18	14-17	≤13
Enrofloxacin	5µg	≥23	17-22	≤16
Tetracycline* *Enterobacteriaceae, Acinetobacter* spp.	30µg	≥15	12-14	≤11
Cephalexin*	30µg	≥18	15-17	≤14
Sulphamethoxazole/ Trimethoprim* *Enterobacteriaceae, Acinetobacter* spp.	25µg	≥16	11-15	≤10

^S, susceptible; I, intermediate susceptibility; R, resistant
*Human-derived zone diameter interpretative standards

Bacteria isolates that acquired non-susceptibility to at least one agent in three or more antimicrobial categories were classified as MDR (Magiorakos *et al.*, 2012).

Results

Out of 65 samples obtained, 16 samples (24.62%) were positive for gram negative bacteria with a total of 22 isolates. Six samples (9.23%) were positive for *A. baumannii*. Figure 2 shows an *Acinetobacter baumannii* isolate on MacConkey agar after 24 hours incubation under 44^{o}C. Among the gram negative bacteria, *A. baumannii* contributed 27.27%, *Achromobacter sp.* contributed 22.72%, *Acinetobacter lowffii* contributed 9.10%, *Enterobacter aerogenes* contributed 9.10%, *Acinetobacter calcoaceticus*, *Pseudomonas aeruginosa*, *Alcaligenes faecalis, Bordetella* sp., *Moraxella* sp., *Hafnia alvei* and *Chromobacter sp.* contributed 4.55% each (Table 3). From Table 4, most *A. baumannii* isolates were contributed by facility 1, 2 and 3 and it is most commonly isolated from stethoscopes (22.2%).

Table 3: Gram negative bacteria isolated from different surfaces.

Types of inanimate objects (No. of surfaces sampled)	Gram negative bacteria isolated	Number of isolates
Door handles (18)	*Acinetobacter baumannii*	1
	Acinetobacter lowffii	2
	Acinetobacter calcoaceticus	1
	Pseudomonas aeruginosa	1
	Achromobacter sp.	1
	Alcaligenes faecalis	1
Examination tables (18)	*Achromobacter* sp.	2
Labcoats (9)	*Acinetobacter baumannii*	1
	Enterobacter aerogenes	2
	Achromobacter sp.	2
Stethoscope (9)	*Acinetobacter baumannii*	3
Weighing scale (9)	*Acinetobacter buamannii*	1
	Bordetella sp.	1
	Moraxella sp.	1
	Hafnia alvei	1
Animal cage (2)	*Chromobacter* sp.	1
Total		22

Table 4: Isolation of *A. baumannii* from different surfaces in veterinary facilities

Objects	**Facilities**				
	1	**2**	**3**	**4**	**Total**
Door handles	1/12	1/2	1/2	0/2	3/18 (16.7)
Examination tables	0/12	0/2	0/2	0/2	0/18 (0%)
Labcoats	0/6	0/1	1/1	0/1	1/9 (11.1)
Weighing Scale	0/6	0/6	0/6	0/6	0/9 (0%)
Stethoscope	2/6	0/1	0/1	0/1	2/9 (22.2%)
Animal cage	0/2	-	-	-	0/2 (0%)
Total	3/44 (6.1%)	1/12 (8.3%)	2/12 (16.7%)	0/12 (0%)	6/65 (9.2%)

Antibiotic susceptibility testing revealed that 15 out of 22 isolates (68.18%) were classified as MDROs whereby they were resistant towards to at least one agent in three or more antimicrobial categories. Most isolates were resistant towards

cephalexin (95.45%), followed by enrofloxacin (59.09%), amoxicillin-clavulanic acid (54.55%), sulphamethoxazole-trimethoprim (54.55%) and tetracycline (50%).

Five out of six *A. baumannii* isolates (83.33%) were classified as MDR after subjected to antibiotic susceptibility test (Table 3). Figure 3 shows an *Acinetobacter baumannii* isolate showing resistance to all antimicrobial tested. From the antibiotic susceptibility profile, all six (100%) *A. baumannii* isolates were resistant to cephalexin, all isolates except for one (83.33%) (Ref. code ST6-2) were resistant to tetracycline and enrofloxacin, three isolates (50%) were resistant towards amoxicillin-clavulanic acid and two isolates were resistant towards sulphamethoxazole-trimethoprim. Antibiotic susceptibility profiles of gram negative isolates are summarized in Table 5.

Table 5: Antibiotic susceptibility profile of gram negative isolates

Ref. code	Bacteria isolates	Antibiotics				
		AMC	ENR	TE	CL	SXT
AWSD3	*Acinetobacter baumannii*	I	R	R	R	I
ST1	*Acinetobacter baumannii*	R	R	R	R	I
ST6-2	*Acinetobacter baumannii*	I	S	S	R	I
BPL2	*Acinetobacter baumannii*	I	R	R	R	R
BPD1-1	*Acinetobacter baumannii*	R	R	R	R	I
BPST	*Acinetobacter baumannii*	R	R	R	R	R
FODD	*Acinetobacter lowffii*	S	S	S	S	S
ISOD2-3	*Acinetobacter lowffii*	S	R	S	R	S
AWSC5	*Bordetella* sp.	R	S	S	R	R
BPL1	*Enterobacter aerogenes*	R	R	I	R	R
VO1	*Enterobacter aerogenes*	S	S	S	R	S
BPD1-2	*Acinetobacter calcoaceticus*	I	R	R	R	S
ISOD2-1	*Pseudomonas aeruginosa*	R	R	R	R	R
ISOD2-2	*Achromobacter* sp.	R	I	S	R	R
LLE1D	*Achromobacter* sp.	R	R	R	R	R
VO6	*Achromobacter* sp.	R	R	I	R	R
VO5	*Achromobacter* sp.	I	I	S	R	R
LLE2C	*Achromobacter* sp.	R	R	R	R	R
BPD1-3	*Alcaligenes faecalis*	I	R	R	R	R
FOC	*Chromobacter* sp.	S	S	S	R	S
AWSC1	*Moraxella* sp.	R	I	S	R	S
WSICU	*Hafnia alvei*	R	I	R	R	R

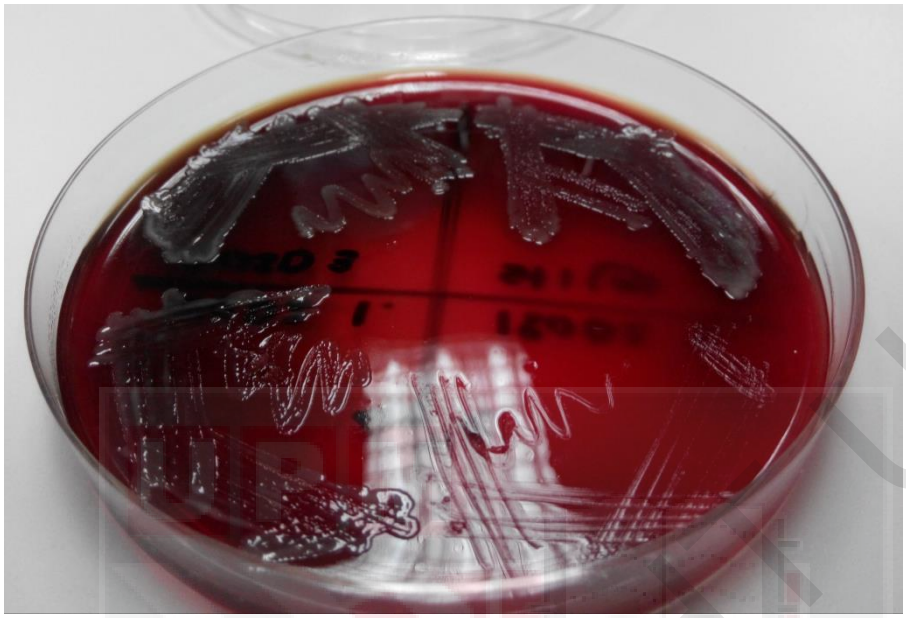

Figure 2: *Acinetobacter baumannii* isolate on MacConkey agar after 24 hours incubation under 44^{o}C.

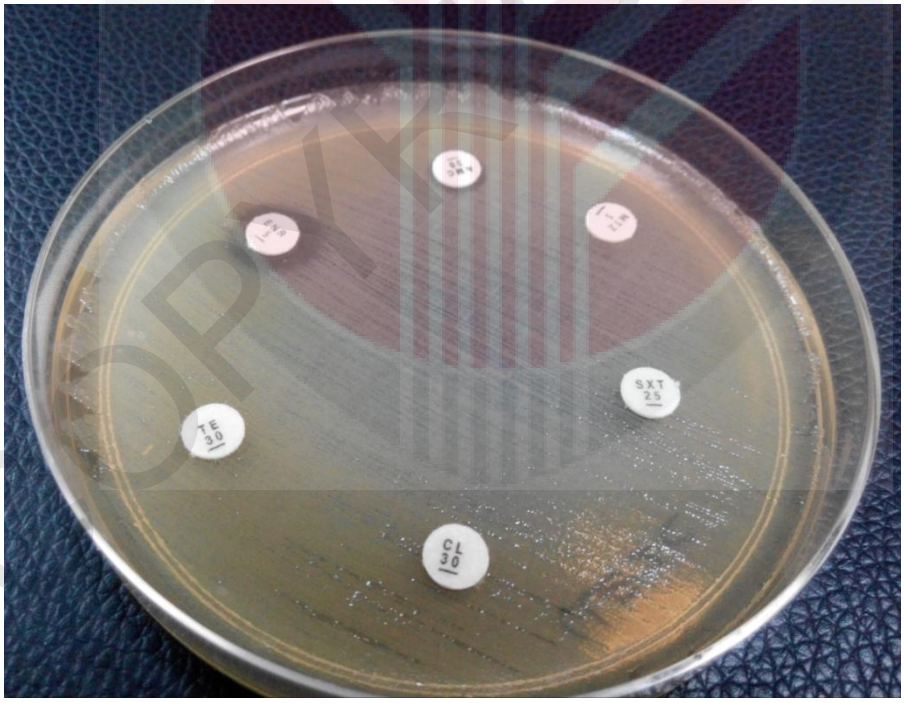

Figure 3: An *Acinetobacter baumannii* isolate showing resistance to all antibiotics tested.

Out of 12 isolates that were previously identified by biochemical test, all were identified as the same bacteria by RapID™ NF Plus except for three isolates (75%) (Table 5). RapID™ NF Plus panel showing typical color reaction for *Acinetobacter* sp. is shown in Figure 4.

Table 5: Comparison of bacteria identification results between biochemical test and RapID™ NF Plus

Ref. code	Biochemical test	RapID™ NF Plus
AWSD3	*Acinetobacter baumannii*	*Acinetobacter* sp.
ST1	*Acinetobacter baumannii*	*Acinetobacter* sp.
ST6-2	*Acinetobacter baumannii*	*Acinetobacter* sp.
BPL2	*Acinetobacter baumannii*	*Acinetobacter* sp.
BPD1-1	*Acinetobacter baumannii*	*Acinetobacter* sp.
BPST	*Acinetobacter baumannii*	*Acinetobacter* sp.
FODD	*Acinetobacter lowffii*	*Acinetobacter* sp.
ISOD2-3	*Acinetobacter lowffii*	Unidentified
BPD1-2	*Acinetobacter calcoaceticus*	*Acinetobacter* sp.
ISOD2-1	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
BPD1-3	*Alcaligenes faecalis*	*Oligella urethralis*
AWSC1	*Moraxella* sp.	*Flavobacterium* 11b

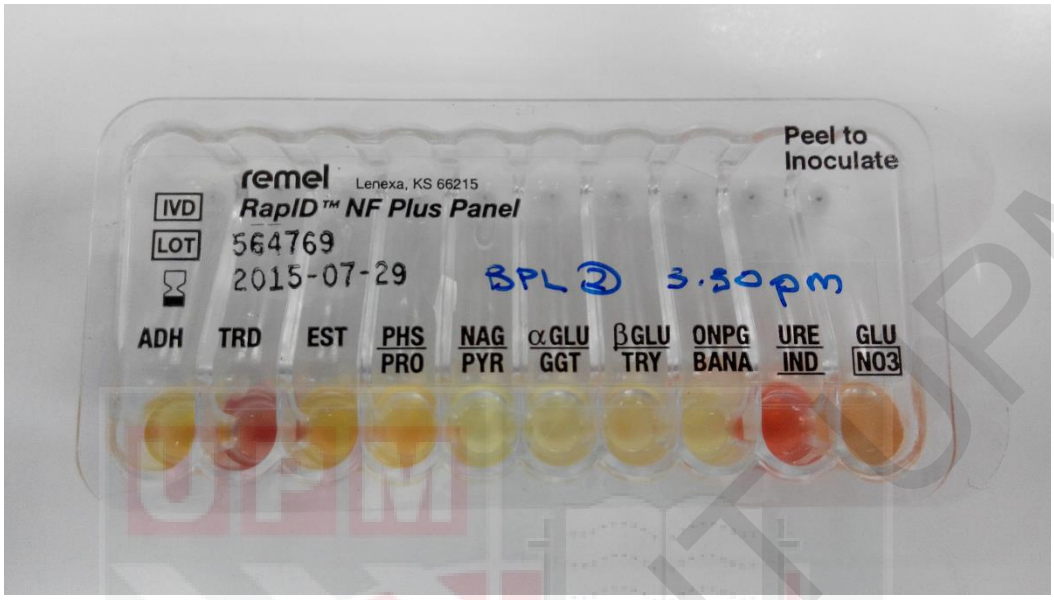

Figure 4: RapID^{TM} NF Plus panel showing typical color reaction for *Acinetobacter* sp.

Discussion

Results showed that six samples (9.23%) were positive for *A. baumannii* while none of the samples was positive for *E. coli*. This imposes that surfaces of inanimate objects can be a source of *A. baumannii* for both human and animals. In this study, *A. baumannii* were identified by using biochemical test and further confirmed up to genus level by using RapID^{TM} NF Plus identification system. According to a study done by Kitch *et al.* (1992), RapID^{TM} NF Plus provides an accurate commercial non-automated method which correctly identified 311 strains out of 345 strains (90.1%) without additional tests. If accurate identification of *A. baumannii* up to species level is required, other identification system such as API 20E System and BBL™ Crystal™ Enteric/Nonfermenter ID Kit can be use (Robinson *et al.*, 1995). Molecular methods of detection are such as pulsed field gel electrophoresis, DNA-DNA hybridization, 16S rRNA gene restriction analysis (ARDRA), amplified fragment length polymorphism (AFLP) (Seifret & Gerner-Smidt, 1995; Dijkshoorn *et al.*, 1998; Janssen *et al*,. 1997).The detection of *A. baumannii* by using molecular method is confirmatory but it is more time consuming.

From the result, no *E. coli* were isolated. The possible reasons for not acquiring any *E. coli* isolates could be due to low prevalence of *E. coli* itself on surfaces swabbed. Therefore this will lower the chance of recovering *E. coli*. Apart from that, according to Elsas *et al* (2011), when *E. coli* are adapted to a niche, they lose the ability to adapt in another. Due to this, enteric *E. coli* that are passed out to the environment may not survive for long. Another possibility of not acquiring

any *E. coli* isolates could be due to an error in sampling, isolation and identification technique. For environmental samples, sample collection are usually done by using moistened sterile swabs and the area to swab should be fixed for example 100cm^2 or 50cm^2 (Public Health Ontario, 2013) and more than one on a large surface. Besides that, sterile gauze pad of a fixed size can be used to swab a larger surface area. Gauze pad also can be further enriched in brilliant green bile 2% to increase the chance of recovering *E. coli* (Barkocy-Gallagher *et al.*, 2002).

In this study, Chromocult® Coliform Agar was used to isolate *E.coli*. This agar contains Tergitol® 7 which inhibit the growth of gram-positive bacteria as well as some gram-negative bacteria without affecting the growth of coliform bacteria and *E. coli.* Therefore Chromocult® Coliform Agar is more selective compared to MacConkey agar in isolation of *E. coli.* Furthermore, *E.coli* appear as dark-blue to violet colonies as they cleave both Salmon-GAL and X-glucuronide. However, growth of gram positive bacteria were observed in most plates and some of these gram positive bacteria were observed to be in light-blue to turquoise, purple, pink and straw color. It can be confusing to differentiate *E. coli* as some colonies are purple in color. Therefore, indole test must be performed to confirm for *E. coli.* Besides that, supplements for *E. coli* can be added as recommended by the manufacturer when excessive growth of gram positive bacteria is observed.

Out of 12 isolates that were previously identified by biochemical test, all were identified as the same bacteria by RapID™ NF Plus except for three isolates (75%). Identification using biochemical test for three of these isolates were less

accurate compared to RapID^{TM} NF Plus. This is because there will be some variation of result between strains of the same species in biochemical testing.

In this study, antibiotic susceptibility testing revealed that 15 out of 22 isolates (68.18%) were classified as MDROs. Most isolates were resistant towards cephalexin (95.45%), followed by enrofloxacin (59.09%), amoxicillin-clavulanic acid (54.55%), sulphamethoxazole-trimethoprim (54.55%) and tetracycline (50%). From the result, we know that the occurrence of multidrug resistant gram negative isolates is quite high which is similar to a study done with human clinical isolates in Assam, India with 50.6% are classified as MDR (Dutta *et al.*, 2014). Cephalexin is a first generation cephalosporin that is active against many gram-positive bacteria and a range of gram-negative bacteria (Bailey *et al*, 1970). However, its resistance among gram negative bacteria is widespread and are rarely recommended for serious gram negative infections (CDC, 2013). Enrofloxacin, amoxicillin-clavulanic acid, sulphamethoxazole-trimethoprim and tetracycline are all broad spectrum antibiotics that are commonly used in small animal practice and are active against both gram positive and gram negative bacteria. Therefore, resistance of these gram negative isolates against drugs mentioned above are significantly alarming. It is recommended that antibiotic susceptibility test should be performed to ensure effective antimicrobial therapy especially in cases of hospital acquired infection.

Five out of six (83.33%) *A. baumannii* isolates were classified as MDR after subjected to antibiotic susceptibility test. From the antibiotic susceptibility profile, all six (100%) *A. baumannii* isolates were resistant to cephalexin. This is

also consistent with a study in Iran with 97% resistant against cephalexin. 65% were resistant to tetracycline. Highest resistant was demonstrated on beta-lactams antibiotic including cephalosporins (Aliakbarzade *et al.*, 2014). For treatment of infections caused by *A. baumannii*, polymyxin B and colistin are considered as the last resort (Zavascki *et al.*, 2007). The ability to aquire resistant determinants easily has made this bacteria to be one of the most troublesome nosocomial pathogen. In a study with 97 clinical isolates, 80% of the isolates were found to be MDR and each strain harboured between one and 17 resistant determinants, and a total of 52 unique resistance determinants or gene families were detected which are known to confer resistance to β-lactam (e.g., $bla_{\text{GES-11}}$, bla_{TEM}, $bla_{\text{OXA-58}}$), aminoglycoside (e.g., *aphA1*, *aacC1*, *armA*), macrolide (*msrA*, *msrB*), tetracycline [e.g., *tet*(A), *tet*(B),*tet*(39)], phenicol (e.g., *cmlA4*, *catA1*, *cat4*), quaternary amine (*qacE*, *qacEΔ1*), streptothricin (*sat2*), sulfonamide (*sul1*, *sul2*), and diaminopyrimidine (*dfrA1*, *dfrA7*, *dfrA19*) antimicrobial compounds. Apart from that, they also found that many of the resistance determinants were found in potentially mobile gene cassettes (Taitt *et al.*, 2014).

As described by CDC (2013), antimicrobial resistant can be prevented by four steps: Preventing infections; improving antibiotic prescribing/ stewardship; developing new drugs and diagnostic tests; tracking and monitoring antibiotic reisstance. Perhaps the most important steps is by improving antibiotic stewardship and it has been defined as the optimal selection, dosage, and duration of antimicrobial treatment that results in the best clinical outcome for

the treatment or prevention of infection, with minimal toxicity to the patient and minimal impact on subsequent resistance (Gerding, 2011).

Conclusion

In conclusion, the occurrence of *Acinetobacter baumannii* in veterinary facilities was 9.23% and 83.33% of *A. baumannii* isolates are MDR. Therefore, surfaces of inanimate objects can be a source of MDR *A. baumannii* for both animals and humans in veterinary health care facilities. For veterinarians, the spread of antibiotic resistant pathogen be prevented by prudent use of antibiotics, use of proper disinfection of surfaces with use of effective disinfectants and monitoring antibiotic resistance trends. For future studies, sampling technique can be improved with larger sample size as the sample size of this study is not representative. Also, antibiotics used in the facility can be related with antibiotic resistance of isolates and lastly, the use of molecular techniques can improve sensitivity of detection of the bacteria of interest.

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