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Journal Articles

OCCURRENCE OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* AND *ESCHERICHIA COLI* IN VETERINARY HEALTHCARE FACILITIES IN KLANG VALLEY, MALAYSIA

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SUMMARY

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 facilities. Surfaces of inanimate objects in health care facilities can serve as 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 in veterinary health care facilities in Klang Valley, Malaysia. In this study, swab samples were taken from 65 surfaces of inanimate objects that included door knobs, examination tables, labcoats, stethoscopes and weighing scales. The swab samples were cultured and all isolates were subjected to antibiotic susceptibility test. The study revealed that the occurrence of *A. baumannii* was 9.23% and 5 out of 6 (83.33%) *A. baumannii* isolates 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 that is of animal and public health concern.

Keywords: Acinetobacter baumannii, Escherichia coli, nosocomial infection, multidrug resistant organisms.

INTRODUCTION

Multidrug-resistant organisms (MDRO) are often involved in hospital associated infection (HAI). 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). As defined by its name, MDROs limits options treatment and may worsen prognosis of patients.

Over the last decade, *Acinetobacter baumannii* have emerged as a significant opportunistic nosocomial pathogen. *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).

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 those suffering from UTI, pyothorax, upper airway obstruction, bloodstream infection and wound infection (Francey *et al.,* 2000).

In a six years study following 505 animal patients with nosocomial bacteraemia, Jerassy *et al*. (2006) concluded that in-hospital mortality in those with *A. baumannii* bacteraemia (57%) was significantly higher than bacteraemia cause by other gram negative organism (31-43%).

Acinetobacter baumannii can be found ubiquitously especially in soil, water, animals and humans (Baumann, 1968; Fournier & Richet, 2006). *Acinetobacter baumannii* is an opportunistic pathogen. Therefore, infections caused by this pathogen are usually found in patients that are ill or immunosuppressed.

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 showed 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 was not statistically different (Jawad *et al.,* 1998). In Malaysia, MDR *A. baumannii* are 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.

Nosocomial *A. baumannii* isolates are mostly multidrug resistant and antimicrobial susceptibility test 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 categorised as

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extensive drug resistant (XDR) with additional resistance to polymyxin B, the drug of "last resort" (Lean *et al.,* 2014).

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 and Lederberg, 2004).

The pathogenic *E. coli* are classified into extraintestinal (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* are 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 (Nataro, 2004).

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 of 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 and Stobbeingringh, 2000).

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

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).

Apart from all the above, Malaysia lacks information regarding the prevalence of these two MDR bacteria especially in veterinary health care settings. Therefore, the objectives of this study were 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.

MATERIALS AND METHODS

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. Sterile swabs pre-moistened with phosphate buffered saline (PBS) were used.

Bacterial isolation and identification

Samples were cultured on MacConkey agar (Oxoid) for isolation of *A. baumannii* and Chromocult® Coliform Agar (CCA) for isolation of *E. coli* and incubated overnight at 37°C. All gram negative bacteria that grew on MacConkey and CCA agar were subcultured after gram staining for 24 hr at 37°C. 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 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 matched all biochemical results were further grown at 41°C and 44°C. The identified *Acinetobacter* spp. were further confirmed up to genus level by using RapID™ NF Plus System (Thermo Scientific). RapID™ NF Plus System is an identification kit based on enzyme technology. It consisted of a clear plastic tray which contained 10 reagent impregnated wells. A suspension of test organism in RapID Innoculation Fluid was used as the inoculum which rehydrated and initiated test reactions. Other gram negative bacteria such as *Pseudomonas aeruginosa, Alcaligenes faecalis,* and *Moraxella* sp. were also tested by using RapID™ NF Plus.

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 scales	9	100cm^3 at the center of the weighing platform
Animal cage	2	100cm^3 at the center of cage floor

Antibiotic susceptibility test

The antibiotic susceptibility test using disk diffusion method was performed on all isolates. The antibiotic agents, concentration of the antibiotic disk used and the antibiotic susceptibility interpretative criteria are summarized in Table 2.

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.* 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 found in facility 1, 2 and 3 and was most commonly isolated from stethoscopes (22.2%).

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*	30µg	≥15	12-14	≤11
Cephalexin*	30µg	≥18	15-17	≤14
Sulphamethoxazole/ Trimethoprim*	25µg	≥16	11-15	≤10

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

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
Stethoscopes (9)	*Acinetobacter baumannii*	3
Weighing scales (9)	*Acinetobacter baumannii*	1
	Bordetella sp.	1
	Moraxella sp.	1
	Hafnia alvei	1
Animal cages (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 scales	0/6	0/6	0/6	0/6	0/9 (0%)
Stethoscopes	2/6	0/1	0/1	0/1	2/9 (22.2%)
Animal cages	0/2	na	na	na	0/2 (0%)
Total	3/44 (6.1%)	1/12 (8.3%)	2/12 (16.7%)	0/12 (0%)	6/65 (9.2%)

na – not available

Antibiotic susceptibility test revealed that 15 out of 22 isolates (68.18%) were classified as MDROs, that is, 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 antibiotics tested. From the antibiotic susceptibility profile, all six (100%) *A. baumannii* isolates were resistant to cephalexin, all isolates except for one (83.33%) were resistant to tetracycline and enrofloxacin, three isolates (50%) were resistant towards amoxicillin-clavulanic acid and two isolates were resistant towards sulphamethoxazole-trimethoprim.

DISCUSSION

The study 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™ NF Plus identification system. According to a study done by Kitch *et al.* (1992), RapID™ NF Plus provides an accurate commercial non-automated method which correctly identified 311 strains out of 345 strains (90.1%) without additional tests. 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. 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. Besides that, using sterile gauze pad of a fixed size a larger surface area and enriched in brilliant green bile 2% may increase the likelihood of recovering *E. coli* (Barkocy-Gallagher *et al.,* 2002).

In this study, antibiotic susceptibility test 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% 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.

The study showed high occurrence of MDR *A. baumannii* and all six (100%) were resistant to cephalexin. This is similar with a study in Iran with 97% resistant against cephalexin and 65% were resistant to tetracycline. Highest resistance 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 acquire resistance 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. A total of 52 unique resistance determinants or gene families were detected which are known to confer resistance to β-lactams, aminoglycoside, macrolide, tetracycline, phenicol, quaternary amine, streptothricin, sulfonamide and diaminopyrimidine 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).

A. baumannii can cause life-threatening nosocomial infections in animals and humans and can limit the treatment options in intensive cares and routine procedures (Francey *et al.*, 2000). Thus, the study showed that surfaces of inanimate objects can be a source of MDR *A. baumannii* in veterinary health care facilities that is of animal and public health concern.

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