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Short communication

Prevalence of *Klebsiella pneumoniae* in surgical practice and laboratory dog husbandry room environments at Rajamangala University of Technology Tawan-ok Veterinary Hospital

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Abstract

Klebsiella pneumoniae is commonly found in environments, causing secondary infections in both human and animals, as well as antibiotic resistance problem. The objective of this study was to determine the prevalence of *K. pneumoniae* contaminated in the environments of surgical practice and laboratory dog husbandry rooms at Animal hospital, Rajamangala University of Technology Tawan-ok during 2019 - 2020. Two-hundred-swabbed samples were collected from these nearby environments and laboratory dogs themselves. Then, all samples were tested for *K. pneumoniae* and antibiotic susceptibility using disk diffusion method. From the results, the total prevalence was 3.5% and the high frequencies were found in three categories: laboratory dogs (35.7%), veterinarian belongings (3.3%) and building structure (2.5%). The antibiotic resistance was also detected including Cephalexin, Ceftazidime and Penicillin G (100%), Gentamicin and Colistin (85.71%), Amikacin (42.86%), and Ciprofloxacin (14.29%). Owning to the fact that laboratory dogs exposed to an external environment leading to bacterial contamination into themself and the husbandry room. Therefore, the hygiene of laboratory dogs and area in husbandry room should be primarily considered. Our study would be the preliminary baseline for the study of antibiotic resistant *K. pneumoniae* contamination in dogs, human, and their environments.

Keywords: Antibiotic resistance, Environment, *Klebsiella pneumoniae*, Laboratory dogs, Prevalence

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INTRODUCTION

Klebsiella is gram negative bacilli in family Enterobacteriaceae, non-motile with encapsulated rod shape (Podschun and Ullmann, 1998). *Klebsiella* spp. are ubiquitous in gastrointestinal tract of various animal species including domestic and wild mammals as well as insects and have been also recovered from foods (Guo et al., 2016). Moreover, they can be found in certain environments such as soil, dust, surface waters, and plants (Seidler et al., 1975; Bagley et al., 1978). *K. pneumoniae* is considered to be one of the most important species among genus Klebsiella causing virulent infection, secondary infection, nosocomial infection and various diseases in human and animals. The clinical signs are as followed pneumonia especially in immunodeficiency patients, chronic respiratory disease, gastrointestinal and urinary tract infections, meningitis, wound infection, abscesses, and septicemia with high mortality rate (Saonuam, 2008; Indrawattana and Vanaporn., 2015; Imai et al., 2019). In animals, *K. pneumoniae* can cause broad spectra of diseases including pneumonia, epidemic metritis, cervicitis in mares and septicemia in foals (Kikuchi et al., 1995), pneumonia and mastitis in bovines (Saishu et al., 2014). According to Munoz et al. (2006)'s study, the reservoir of this bacterial species was in cattle feces related to the occurrence of mastitis. Moreover, *Klebsiella* spp. could be isolated from most bedding samples. Kreunumkum et al. (2016) revealed that 14.28% of bacteria determined from pet wounds was *Klebsiella* spp. In addition, there was a report of *Klebsiella* spp. infection in a bull terrier dog's hard palate after surgical repair of cleft hard palate (Stevanovic et al., 2019).

K. pneumoniae is not only clinically significant, but also gaining much more concern on public health. It is considered as one of the opportunistic pathogens showing increasingly frequent acquisition of resistance to antibiotics (Effah et al., 2020; Haenni et al., 2011) especially multidrug resistance (MDR). The most frequently found resistance is extended-spectrum β-lactamase (ESBL) producing *K. pneumoniae* (Wareth and Neubauer, 2021; Kuan et al., 2016). The majority of researches in the present is usually focusing on only other members of Enterobacteriaceae such as Salmonella and *Escherichia coli*, while the studies of *K. pneumoniae* especially in veterinary medicine are limited. The risk of human infection related to animal contact and food consumption is not well understood. However, the transmission pathways between humans, animal hosts, and the environment are currently a subject of active discussion.

Therefore, the objective of this study was to determine the prevalence of *K. pneumoniae* contaminated in the environments of surgical practice and trial dog husbandry rooms, which is a part of surgical practice room at Animal hospital. This part of the hospital has to be cleaned and aseptic. In addition, our study was also to determine what can be the possible sources of *K. pneumoniae*, so that the prevention and control of bacterial contamination would be essentially considered.

MATERIALS and METHODS

Sample collection

This study was a descriptive study by collecting 200 samples swabbed from different six categories including laboratory dogs (n=14), laboratory dogs' equipment (n=20), building structures (n=40), operative equipment (n=70), staff's hand (n=26), and veterinarian belongings (n=30) in surgical practice and trial dog husbandry rooms at Animal Hospital of Rajamangala University of Technology Tawan-ok, Chonburi province, Thailand during December, 2019 to August, 2020.

Klebsiella pneumoniae isolation

All samples were cultured on Tryptic soy broth (TSB) and incubated at 37 and 44^{o}C for 24-48 hours (Schiraldi and De Rosa, 2014). Then the turbid TSB was transferred to culture on MacConkey agar and incubated in the same condition above. After that, a single typical (pink or dark pink) colony was transferred from MacConkey agar to conduct for gram staining and biochemical tests which applied from Podschun and Ullmann (1998); Josy et al. (2018); Saonuam (2008); and Munoz et al. (2006). All confirmed *K. pneumoniae* isolates were properly recorded and stored for further analysis.

Antibiotic susceptibility testing

All confirmed *K. pneumoniae* isolates were taken to antibiotic sensitivity test by disk diffusion method of Kirby-Bauer (Clinical and Laboratory Standards Institute: CLSI, 2012), and used data from Pruekprasert et al. (2006), Puncharat et al. (2015) and Kreunumkum et al. (2016) for selected antibiotic disks including Amikacin (AMK; 30 µg), Ceftazidime (CAZ; 30 µg), Cephalexin (CFX; 30 µg), Ciprofloxacin (CIP; 5 µg), Colistin (CST; 10 µg), Gentamicin (GEN; 10 µg), Imipenem (IPM; 10 µg), and Penicillin G (PCN; 10 units). *E. coli* ATCC 25922 was used as reference strain to measure sensitivity. The isolates with resistance to three or more classes of antibiotics were classified as multidrug resistance (MDR).

Statistical analysis

The statistical analysis was applied to determine the prevalence of *K. pneumoniae* derived from three categories (laboratory dogs, veterinarian belongings and building structures) for the most variables. The analysis with likelihood ratio test and Fisher's exact test, using R version 3.1.2 (R foundation for statistical computing, Vienna, Austria) were applied. Results were reported as odds ratios (OR) with its associated 95% confidence interval (CI), and a P-value of less than 0.05 was considered as significance.

RESULTS

From all 200 samples swabbed from surgical practice and trial dog husbandry rooms at Animal hospital, the overall prevalence of *K. pneumoniae* was 3.5% (7/200). The most frequencies were found in laboratory dogs 35.7% (5/14), veterinarian belongings 3.3% (1/30), and building structures 2.5% (1/40), respectively (Table 1). The samples collected from other sources including laboratory dogs' equipment, staff's hand, and operative equipment were negative for *Klebsiella* spp. The laboratory dogs showed the highest prevalence with 5 samples out of 14 including saliva (2/4) and laboratory dog bodies (3/10). The percent prevalence of laboratory dogs' saliva was 50% with OR = 38.2, 95% CI = 4.442 - 246.164, and P-value = 0.006. While laboratory dogs' bodies had the prevalence of 30% with OR = 19.9, 95% CI = 3.727 - 155.367, and P-value = 0.003. The OR of *K. pneumoniae* prevalence in sewer pipes samples was 3.4, while this value in shoes used in surgical practice room was 1.53 compared to others factors of source (Table 1).

Table 1 Univariate analysis of sources of contamination associated with the prevalence of *K. pneumoniae*

Sources of contamination[a]	Prevalence (%)	OR	95% CI of OR	P-value[b]
Building structures	1/40 (2.5)	0.7	0.076-5.627	1.000
Cage floors	0/30 (0.0)	0.0	0.000	0.000
Sewer pipes	1/10 (10.0)	3.4	0.373-3.407	0.306
Laboratory dogs	5/14 (35.7)	51.1	8.696-411.611	0.000
Saliva	2/4 (50.0)	38.2	4.442-246.165	0.006
Dogs' bodies	3/10 (30.0)	19.9	3.727-155.367	0.003
Veterinarian belongings	1/30 (3.3)	0.9	0.109-5.775	1.000
Shoes used in surgical practice room	1/20 (5.0)	1.5	0.174-11.91	0.527
Lockers	0/10 (0.0)	0.0	0.000	0.000

[a] The negative samples for *Klebsiella* spp. were not included in the present table.
[b] The Fisher's exact test statistic at P-value < 0.05 of significance.

According to antibiotic sensitivity test, all *K. pneumoniae* isolates were resistant to Cephalexin, Ceftazidime and Penicillin G (7/7). Six out of seven *K. pneumoniae* isolates (85.71%), except one isolate from dogs' bodies, were resistant to Gentamicin and Colistin. The *K. pneumoniae* isolates from shoes used in surgical practice room, sewer pipe and dogs' saliva were resistant to Amikacin (3/7) which considered as 42.86% of resistance. Only one isolate from sewer pipe (T2-1) was resistant to Ciprofloxacin (1/7) which as 14.29% of resistance. However, all *K. pneumoniae* isolates were susceptible to Imipenem (7/7) which as 100% of susceptibility (Table 2). All seven *K. pneumoniae* isolates in this study were classified as MDR. The predominant resistant patterns (R-patterns) were tabulated in Table 3. We found that the most frequent patterns were CAZ CFX CST GEN PCN (42.86%) from laboratory dogs, followed by AMK CAZ CFX CST GEN PCN (28.57%) from laboratory dogs and shoes used in surgical practice room.

Table 2 Percentage of resistance by types of antibiotics.

Antibiotics[a]	*K. pneumoniae* isolates[b]							Percentage of resistance
	K10-1	T2-1	OS4-1	OS8-1	OS8-2	I4-1	I4-2	
AMK	R	R	S	S	S	R	S	42.86
CAZ	R	R	R	R	R	R	R	100.00
CFX	R	R	R	R	R	R	R	100.00
CIP	S	R	S	S	S	S	S	14.29
CST	R	R	R	R	S	R	R	85.71
GEN	R	R	R	R	S	R	R	85.71
IPM	S	S	S	S	S	S	S	0.00
PCN	R	R	R	R	R	R	R	100.00

[a] Amikacin (AMK), Ceftazidime (CAZ), Cephalexin (CFX), Ciprofloxacin (CIP), Colistin (CST), Gentamicin (GEN), Imipenem (IPM), and Penicillin G (PCN)
[b] K10-1 = isolate from shoes used in surgical practice room; T2-1 = isolate from sewer pipe; OS4-1, OS8-1 and OS9-2 = isolates from dogs' bodies; I4-1 and I4-2 = isolates from dogs' saliva.

Table 3 Predominant R-patterns of *K. pneumoniae* isolates

Predominant R-patterns[a] (n)	*K. pneumoniae* isolates[b]	Percentage
CAZ CFX CST GEN PCN (3)	OS4-1, OS8-1, I4-2	42.86
AMK CAZ CFX CST GEN PCN (2)	K10-1, I4-1	28.57
AMK CAZ CFX CIP CST GEN PCN (1)	T2-1	14.29
CAZ CFX PCN (1)	OS8-2	14.29

[a] Amikacin (AMK), Ceftazidime (CAZ), Cephalexin (CFX), Ciprofloxacin (CIP), Colistin (CST), Gentamicin (GEN), Imipenem (IPM), and Penicillin G (PCN)
[b] K10-1 = isolate from shoes used in surgical practice room; T2-1 = isolate from sewer pipe; OS4-1, OS8-1 and OS9-2 = isolates from dogs' bodies; I4-1 and I4-2 = isolates from dogs' saliva.

DISCUSSION

The aim of this study was to determine the prevalence of *K. pneumoniae* and the source of contamination by collecting the animal and environmental samples from surgical practice and trial dog husbandry rooms at Animal hospital. According to Table 1, the prevalence of *K. pneumoniae* in laboratory dogs which as 35.7% (5/14) related to the previous study conducted by Kreunumkum et al., (2016) at a pet hospital in Chiang Mai province, Thailand. They found that the prevalence of *Klebsiella* spp. isolated from various types of wounds including surgical wound, bite wound, abscess and cancer in dogs and cats was 14.28% (4/28) with multiple antimicrobial resistance. In some cases, the present of *Klebsiella* spp. can interfere the wound healing process which showed in a case report of 8-month female Bull Terrier with surgical wound of hard palate (Stevanovic et al., 2019). However, *Klebsiella* spp. infections are frequently proceeded by gastrointestinal colonization and the gastrointestinal tract is believed to be the most important reservoir for transmission of this bacteria (Schiraldi and De Rosa, 2014). Indeed, *K. pneumoniae* can be recovered from other clinical specimens such as sputum, urine and wound-pus (Puncharat, 2015), as well as clinical samples from companion and farm animals (Kuan et al. 2016; Wareth and Neubauer, 2021). In accordance with our study, we also found the prevalence of this bacteria in building structures especially sewer pipes as 10% (1/10) (Table 1), correlated to the data documented by Wareth and Neubauer (2021) that *K. pneumoniae* was found in raw waste water and water specimens. Contrast to our result of sewer pipes which collected in limited number of samples in a small area, the study by Pongparit et al. (2013) reported 76% of *K. pneumoniae* prevalence in wastewater around Bangkok and metropolitan area.

Turning to consider the source of contamination (Table 1), the laboratory dogs had 51.1 times of the odds having *K. pneumoniae* compared to other sources of contamination. Focusing on dogs' saliva and bodies, it can be concluded that the prevalence of *K. pneumoniae* in this study was significantly related to the laboratory dogs. Other interesting sources of contamination were sewer pipes and shoes used in surgical room, even they were just a trending. From these results, it might be assumed that the contamination of this bacteria into environments of surgical practice room might come from the laboratory dogs which could access to outside of building and this bacteria could be disseminated by students and staff working in that surgical room.

From the results of antibiotic sensitivity test (Table 2), Amikacin, Ciprofloxacin and Imipenem had the percentage of resistance as 42.86, 14.29 and 0% respectively. These percentages of latter three antibiotics were quite according to the study of Pruekprasert et al. (2006) that the strains of *K. pneumoniae* were resistant to Amikacin and Ceftazidime, but were susceptible to Imipenem. Kreunumkum et al. (2016) reported that *Klebsiella* spp. isolated from cat wounds were resistant to Cephalexin and other β-lactams which correlated to our study. In accordance with the research conducted by Josy et al. (2018), *K. pneumoniae* isolates derived from animal handler samples were 100% resistant to Penicillin. Furthermore, Puncharat et al. (2015) revealed that ESBL-producing *E. coli* and *K. pneumoniae* had less than 50% susceptibility to Gentamicin which was in agreement to our result. Those bacteria are in the same family, Enterobacteriaceae, containing plasmids harbored different resistance genes resulting in the horizontal gene transfer of antimicrobial resistance (Pornsukarom and Thakur, 2017; Chatzopoulou et al., 2018). When considering Table 3, all *K. pneumoniae* isolates in this study had trend to MDR which can be caused global public health problem. Moreover, the isolates from dogs' saliva and shoes in surgical room shared the same R-pattern. This result provided a strong evidence of antimicrobial resistance dissemination. Interestingly, one MDR *K. pneumoniae* isolate (T2-1) recovered from sewer pipe was resistant to Ciprofloxacin. This antibiotic is commonly used in human clinical treatment (Conley et al., 2018). The presence of Ciprofloxacin resistance would be a sign of becoming public health threat. Thus, it can be assumed that sewer pipe as intermediate environment for antimicrobial resistant transmission. The further analysis for mechanism of resistance should be promoted.

However, in Thailand, researches on *Klebsiella* spp. in environment are few and still limited. According to our study, *K. pneumoniae* was found in dogs' saliva, dogs' bodies, also in sewer pipes at trial dog husbandry room, and shoes at the surgical practice room. It can be assumed that animal hospital staffs have to pay more attention on hygiene management and sanitation in order to decrease the prevalence of this bacteria and also to prevent chances of nosocomial infections in the future. Nonetheless, the result of this study can be used as a baseline information for other studies such as antimicrobial susceptibility test or lay down measures of hygiene management protocol for laboratory dogs.

CONCLUSION

K. pneumoniae is ubiquitously found in nature, human or animals' body and also in the environment of working places such as surgical practice room which should be clean and aseptic. We found that these practice and laboratory dog husbandry rooms showed the prevalence of this bacteria, and factor causing this value was from laboratory dog itself. Therefore, giving importance of hygienic management should be considered. Furthermore, this study also revealed the presence of MDR *K. pneumoniae*, which related to the rational drug use and recent public health problem.

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AUTHOR CONTRIBUTIONS

KR, TC, KP, VJ collected samples; VJ, KN operated in laboratory. KP, VJ, SP. collected and categorized the data. KN and SP conceived and designed analysis. KP, VJ and SP analyzed the data. KR and TC artwork and table designed; KN and SP wrote the manuscript.

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