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PREVALENCE AND MOLECULAR DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM DOGS AND CATS IN DHAKA CITY

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ABSTRACT

Pet (dog and cat) animal numbers have substantially increased in modern society. There is concern over transmission of Staphylococcal infection including methicillin-resistant *Staphylococcus aureus* (MRSA) between animals and humans. The objective of the present study was to determine the prevalence of MRSA from apparently healthy and diseased dog and cat of different veterinary Hospital, clinics and pet animal market in Dhaka city. Samples collected for detection of MRSA were nasal swab, pus and wound swab. Among the 93 samples, 40.86% (n=38/93) were confirmed as positive for *S. aureus* and4.30% (n=4/93) as MRSA. The detection of MRSA was confirmed phenotypically and also by PCR targeting *mec A*gene specific for MRSA. All isolates isolated *S. aureus* were coagulase positive and hence pathogenic. Antibiogram study showed that all these isolates were sensitive to vancomycin and tetracycline. The overall prevalence of MRSA was higher in dog (4.91%) compared to cat (3.13%). The highest prevalence of MRSA (5.88%) was recorded in samples collected from K ataban Pet Animal Market, Dhaka. On sample basis MRSA was higher in nasal swab compared to pus and wound swab. On age basis, the prevalence of MRSA was higher in younger animal compared to older animal. The highest prevalence of *S. aureus* was found in diseased dog and highest prevalence of MRSA was found in diseased cat. None of the healthy cat was found positive for MRSA. Present study emphasizes that dogs and cats may act as a possible reservoirs for transmission of MRSA to human.

Keywords: Dog, cat, methicillin-resistant *Staphylococcus aureus* (MRSA), prevalence, *mecA,* PCR.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a specific strain of the *S. aureus* that has developed resistance against the beta-lactam class of antibiotics, which includes penicillin and derivates such as methicillin, oxacillin, and amoxicillin (Foster, 1996).The genetic basis of MRSA is due to the presence of *mecA* gene encoding low affinity penicillin-binding protein 2A (PBP2A), that act as a surrogate trans-peptidase in the presence of high concentrations of β-lactam antibiotics which inactivate the four high-affinity PBPs native to *S. aureus* (de Jonge and Tomasz, 1993).

MRSA was first discovered in the UK in 1961 but it is now widespread across the globe. Several studies have been done in Bangladesh especially in human to see the burden of MRSA infection (Haque *et al.* 2011; Islam *et al.*, 2011) reported a prevalence of 43·7% MRSA in clinical samples. In another study Islam *et al.* (2011) detected gene *mecA* responsible for resistance against methicillin by PCR from 25.0% human clinical samples. MRSA are also found in animal. In 1972, MRSA was found in milk from Belgian cows with mastitis. There are reports on the detection of MRSA from dogs, cats, sheep, cattle, horses, rabbits, seals, cockroach, guinea pig and chinchilla (Morgan, 2008; Chandrasekaran *et al.*, 2014; Gulani *et al.*, 2016; Islam *et al.*, 2016). The *mecA* gene has been detected from Staphylococcus isolated from dairy cattle mastitis (Rahman *et al.*, 2005).

MRSA are serious public health concern since they could not be treated effectively with many antibiotics easily. Lee *et al.* (2003) reported the potentiality of transmission of MRSA from food animal to human. Across the globe, an estimated 2 billion people carry some form of *S. aureus*; of these, up to 53 million (2.7% of carriers) are thought to carry MRSA. It has been suggested that zoonotic transmission of MRSA can takes occur between pet owners and their pets (VAN Balen *et al.*, 2017). Several works have been carried out in abroad on the prevalence of MRSA in dog and cat (VAN Balen *et al.*, 2017; Karkaba *et al.*, 2017). In Bangladesh, MRSA has been isolated from raw milk samples of cow (Jahan *et al.*, 2015). However, no work has been reported in Bangladesh describing the isolation of MRSA from healthy and diseased Dog and Cat in Dhaka city. In the present study, we investigated the prevalence of MRSA in dog and cat used as pet animal in Dhaka city and their zoonotic significance.

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MATERIALS AND METHODS

Sampling areas

The pet dogs and cats used for this study were available at the Central Veterinary Hospital (CVH), Dhaka; Gulshan Pet Clinic, Dhaka; Kataban Pet Animal Market, Dhaka. Dhaka, the capital city of Bangladesh.

Collection of samples

Nasal swab, pus and wound swab sample were collected from dog and cat pet clinics. A total of 93 swab samples were collected. All of these pets were Hybrid and cross breed with average age of 1-3 years old. Detail of the sampling data is available in Table 1.

Table 1. Name of collection points with the number of samples collected from each of the points

Sample collection area	No. of samples				Total
	Dog		Cat		Dog and Cat
	Healthy	Diseased	Healthy	Diseased	
Central Veterinary Hospital (CVH), Dhaka	13	34	07	12	66
Kataban Pet Animal Market, Dhaka	08	02	06	01	17
Gulshan Pet Clinic, Dhaka	01	03	02	04	10
Diseased or Healthy	22	39	15	17	93

Isolation of *Staphylococcus aureus*

Isolation of *S. aureus* was done using standard culture, staining and biochemical test, as described by Islam *et al*. (2007) and Jahan *et al.* (2015).

PCR for detection of *mecA* gene

The genomic DNA from *S. aureus* was extracted by boiling method as described by Begum *et al.* (2016). Detection of *mecA* was done by PCR using primers (Table 2) described by Bennimath *et al.* (2011).

Each PCR was done in 25 µl reaction that consisted of 5µl genomic DNA, 12.5 µl PCR master mixture (Promega) 1 µl of each of the two primers and 5.5 µl of *nuc*lease free water. The PCR amplification was done by initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing temperature of primers was 55°C for 45 seconds and extension at 72°C for 1.5 minutes. The final extension was conducted at 72°C for 3 minutes. At the end of PCR, the PCR produces were run on a 2% agarose gel, stained with ethidium bromide and photographed using a Gel documentation system (BioRad).

Table 2. Primers used in PCR for the detection of methicillin resistant *S. aureus*

Primer name	Primers sequence	Product size(bp)	Reference
mecA/F	5'-GTGGAATTGGGCAATACACC-3'	533	Bennimath
mecA/R	5'-AGTTCTGCAGTACCGGAT -3'		*et al. (*2011)

Antibiotic sensitivity test

Antibiotic sensitivity test of the isolated *S. aureus* was performed using Mueller Hinton agar as described by Kirby-Bauer disc diffusion method (Begum *et al.*, 2016). Inhibition of zone diameters was measured and values obtained from the National Committee on Clinical Laboratory Standards were used to interpret the results obtained. *S. aureus* isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic based on the standard interpretation table updated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2011). The antibiotics disc used were methicillin (oxacillin) -10 µg / disc, vancomycin-30 µg / disc and tetracycline 30 µg / disc.

RESULTS

A total of 93 samples were collected and examined for isolation and identification of *S. aureus* to detect MRSA. Out of the 93 swab samples, 38 (40.86%) isolates were identified as *S. aureus* on the basis of morphology, staining and cultural characteristics on blood agar (BA) and Mannitol Salt Agar (MSA). Grams staining identified them as grape like clusters. In BA they produced characteristics β-hemolysis (Figure 1) and on MSA they fermented mannitol and produced characteristics yellowish colonies (Figure 2).

Detection of MRSA based on phenotype

Isolated *S. aureus* were subjected to antimicrobial resistance profile assessment for phenotypic investigation of MRSA. MRSA is identified by assessing zone of inhibitions with oxacillin ≤ 10 mm (CLSI, 2011) (Figure 3). Among the 38 *S. aureus* 4 was found resistant to oxacillin (methicillin).

Confirmation of MRSA by PCR targeting *mecA* gene

In order to develop a quick and reliable confirmatory diagnostic test using molecular method for MRSA PCR was performed. At the genomic level, the optimized PCR assay was able successfully to amplify the target *mecA* gene expected size 533 bp fragment from the genomic DNA. The presence of methicillin resistant *S. aureus* was confirmed in two samples by amplification of the approximately 533 bp DNA fragment (Figure 4).

Prevalence of *Staphylococcus aureus* and MRSA in dog and cat

The overall prevalence of *S. aureus* was higher in dog (42.62%) compared to cat (37.50%) (Table 3, Figure 5). Similarly the overall prevalence of MRSA was higher in dog (4.91%) compared to cat (3.13%) (Table 3).Prevalence of *S. aureus* in healthy dog and cat was 22.73% and 26.67% respectively, where diseased dog and cat was 53.85% and 47.05% respectively (Table 3). The prevalence of MRSA in healthy dog was 4.55% while none of the healthy cat was found positive for MRSA. The prevalence of MRSA in diseased dog and cat was 5.13% and 5.88% respectively. The highest prevalence of *S. aureus* was found in diseased dog and highest prevalence of MRSA was found in diseased cat (Table 4).

Table 3. Prevalence of *S. aureus and* MRSA between dog and cat

Species	Tested sample	No. of *S. aureus* (%)	No. of positive MRSA	Prevalence of MRSA (%)
Dog	61	26 (42.62%)	3	4.91%
Cat	32	12(37.50%)	1	3.13%
Total	93	38 (40.86%)	4	4.30%

Table 4. Prevalence of *Staphylococcus aureus* and MRSA in dog and cat according to health status

Species		Tested sample	No. of *S. aureus* (%)	No. of positive MRSA	Prevalence of MRSA (%)
Dog	Healthy	22	5 (22.73%)	1	4.55%
	Diseased	39	21 (53.85%)	2	5.13%
Cat	Healthy	15	4 (26.67%)	0	0.00%
	Diseased	17	8 (47.05%)	1	5.88%
Total		93	38 (40.86%)	4	4.30%

Prevalence of *S. aureus* and MRSA according to type of samples

Samples analyzed in this study were nasal swab, wound swab and pus. Prevalence of *S. aureus* and MRSA in nasal swab, wound swab, pus was 30.99%, 64.28%, 87.5% and 4.23%, 0.00%, 12.5% respectively (Table 5).

Prevalence of *S. aureus* and MRSA in dog and cat according to source of samples

The prevalence of *S. aureus* and MRSA based on source of samples of Dhaka city is shown in Table 6. Highest prevalence of *S. aureus* was found in Central Veterinary Hospital (CVH), Dhaka (46.97%). Prevalence of *S. aureus* in Kataban Pet Animal Market, Dhaka and Gulshan Pet Clinic, Dhaka were (23.53%) and (30%) respectively. The highest prevalence of MRSA was recorded Kataban Pet Animal Market, Dhaka (5.88%)

compared to Central Veterinary Hospital (CVH), Dhaka (4.55%) Kand Gulshan Pet Clinic, Dhaka (0.00%) shown in Table 11.

Table 5. Prevalence of *S. aureus and* MRSA indifferent types of samples

Collected samples	Total samples (n=93)		Total no. of positive *S. aureus* (n=38)	Total no. of positive MRSA (n=4)
	Dog (61)	Cat (32)		
Nasal swab	47 (77.04%)	24 (75%)	22 (30.99%)	3 (4.23%)
Wound swab	9 (14.75%)	5 (15.63%)	9 (64.28%)	0 (0.00%)
Pus	5 (8.20%)	3 (9.38%)	7 (87.5%)	1 (12.5%)

Table 6. Prevalence of *S. aureus* and MRSA in dog and cat according to source of samples

Sample collection area	Tested sample	No. of positive *S. aureus* (%)	No. of positive MRSA	Prevalence of MRSA (%)
Central Veterinary Hospital (CVH), Dhaka	66	31 (46.97%)	3	4.55%
Kataban Pet Animal Market, Dhaka	17	4 (23.53%)	1	5.88%
Gulshan Pet Clinic, Dhaka	10	3 (30%)	0	0.00%
Total	93	38 (40.86%)	4	4.30%

Prevalence of *S. aureus* and MRSA in dog and cat according to age

Prevalence of *S. aureus* and MRSA in different age of dog and cat are shown in Table 7. Prevalence of *S. aureus* was highest in ≥1.5 year dog 42.86% than <1.5 year dog 42.42% and prevalence of MRSA in <1.5 year dog was 6.06%, which was higher than ≥ 1.5year dog 3.57%. On the other hand, Prevalence of *S. aureus* was highest in < 1year cat 42.10% than ≥ 1 year cat 30.77% and prevalence of MRSA in <1 year cat was 5.26%, which was higher than ≥1 year cat 0.00%.

Table 7. Age wise prevalence of *S. aureus* and MRSA in dog and cat

Age		Tested sample	No. of *S. aureus* (%)	No. of positive MRSA	Prevalence of MRSA (%)
Dog	≥ 1.5 year	28	12 (42.86%)	1	3.57%
	< 1.5 year	33	14 (42.42%)	2	6.06%
Cat	≥ 1 year	13	4 (30.77%)	0	0.00%
	< 1year	19	8 (42.10%)	1	5.26%
Total		93	38 (40.86%)	4	4.30%

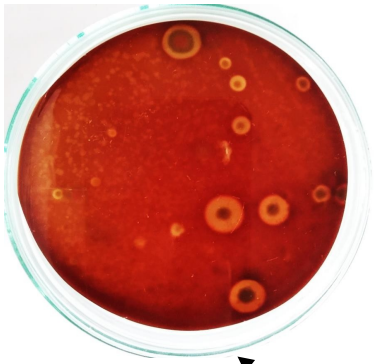

Figure 1. β-hemolysis produced by *S. aureus* on 5% sheep blood agar

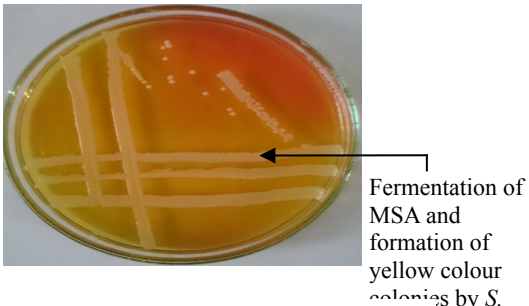

Figure 2. Cultural properties of *S. aureus* onto MSA media

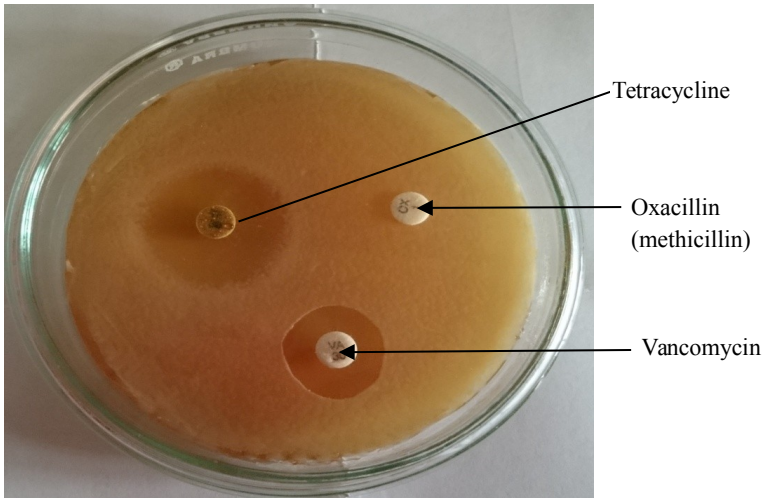

Figure 3. Methicillin (oxacillin),vancomycin and tetracycline sensitive pattern of *S. aureus*

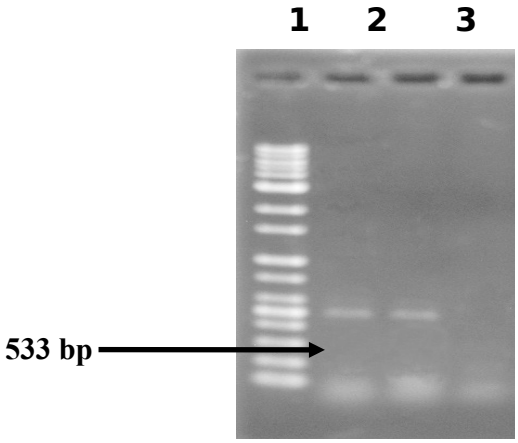

Figure 4. Molecular detection of methicillin resistant *Staphylococcus aurous* by PCR
(Lane 1: 1kb DNA ladder, Lanes 2-3: Tested samples, Lane 4: Negative control)

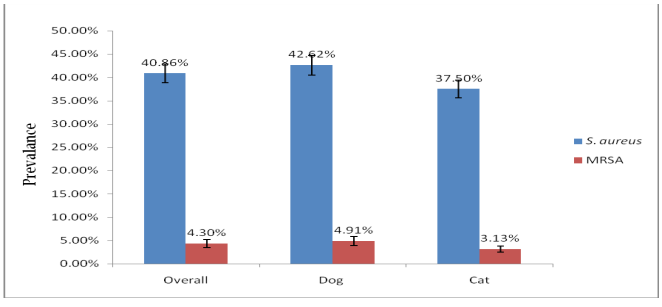

Figure 5. The overall prevalence of *S. aureus* and MRSA in dog and cat

DISCUSSION

MRSA has emerged as a significant public health problem both in human and veterinary medicine worldwide. Among the Gram-positive pathogens, *S. aureus* continues to cause skin and soft tissue infections in human and animal including hospitalized patients (Joshi *et al.*, 2011). MRSA got zoonotic importance when scientists suggested the possibility of dog and cat serving as reservoirs for human MRSA infection.

In this study, we investigated the prevalence MRSA in pet dogs and cats of Dhaka city. Among the 93 samples analyzed (61 from pet dogs and 32 from pet cats) 38 (40.86%) was found positive for *S. aureus*. All these 38 isolates were considered as pathogenic since all of them were coagulase positive, although many of these were isolated from healthy animal. Among these 38 *S. aureus,* four (4.30%) isolates were found to show resistant to oxacillin and considered as MRSA. But interestingly all these isolates were found sensitive to vancomycin and tetracycline, suggesting that vancomyc in and tetracycline could be the preferred antibiotic to treat the infection caused by these *S. aureus* including the MRSA. In fact previously Oberoi *et al.* (2012) and Lee (2003) also showed the susceptible of MRSA to vancomycin. But Tiwari and Sen (2006) reported resistance of MRSA against vancomycin resistance. According to a conference report submitted by Rahman (2015) the prevalence of MRSA was found 5.12% in Dog in Dhaka city, which is similar to the finding we have reported here.

The highest prevalence of MRSA was recorded Kataban Pet Animal Market, Dhaka (5.88%). People bring their pet in the Kataban Pet Animal Market for sell. It was not unexpected to see the highest prevalence of MRSA in pet in this market, because it is a mixing place of different types of animal, allowing cross contamination of MRSA positive animal with MRSA negative animals.

To the best of our knowledge, this is the first report describing prevalence of MRSA in healthy and disease Dog and Cat in Dhaka city in Bangladesh. Presence of MRSA in pet animal as detected in this study is alarming, because MRSA are zoonotic in nature. The pet may act as potential reservoirs for transmission for MRSA to human. Attention therefore should be taken for early detection of MRSA and application of suitable antibiotic to cure them.

CONCLUSIONS

MRSA is a health problem both for animal and human across the globe. Pets (dog and cat) animal may act as a reservoir and source of MRSA for human. In this study we determined the prevalence of MRSA in apparently healthy and diseased dog and cat in Dhaka city. Among the 93 samples, 40.86% (n=38/93) were confirmed as positive for *S. aureus* and 4.30% (n=4/93) for MRSA. All *S. aureus* isolates were pathogenic in nature as revealed by coagulase test. Present study suggests that dogs and cats may act as possible reservoirs for transmission of MRSA to human.

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