ISSN No:-2456-2165

Epidemiological Investigation, Prevalence & Antibiogram Study of Potential Zoonotic Bacterial Pathogens of Household Pets at Dinajpur District of Bangladesh

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Abstract:- A cross sectional experimental study was conducted to ascertain the prevalence of potential zoonotic bacterial pathogen in household pet animals (dog, cat and rabbit). The study was done in selected areas of Dinajpur district during the period of July 2016 to June 2017. A total of 79 pet animals comprising of 50 (63.29%) dogs; 10 (12.66%) cats and 19 (24.05%) rabbits were observed and samples were collected considering different socio demographic variable. The organisms were isolated by using standard microbiological techniques. A total of 404 samples were examined and 7 isolates of potential zoonotic bacterial pathogens were isolated in pet animals. The overall prevalence of zoonotic pathogens in dogs out of 250 samples, was 15.2% *E. coli*; 10.8% *Klebsiella* spp; 12% *Salmonella* spp; 8% *Proteus* spp; 8.8% *Pseudomonas* spp; 12% *Staphylococcus* spp; 10.4% *Streptococcus* spp respectively. The overall prevalence of zoonotic pathogens in cats out of 40 samples was 17.5% *E.coli*; 10% *Klebsiella* spp; 12.5% *Salmonella* spp; 10% *Proteus* spp; 10% *Pseudomonas* spp; 15% *Staphylococcus* spp; 15% *Streptococcus* spp respectively. The overall prevalence of zoonotic pathogens in rabbits was 12.28% *E.coli*; 7.89% *Klebsiella* spp; 9.65% *Salmonella* spp; 4.38% *Proteus* spp; 1.75% *Pseudomonas* spp; 6.14% *Staphylococcus* spp; 6.14% *Streptococcus* spp respectively. The prevalence of bacterial zoonotic pathogen between pet animals and housing system, hygienic condition, vaccination were statistically significant (P≤ 0.01). On the other hand the prevalence of bacterial zoonotic pathogen between pet animals and age, sex, breed, body weight, diet, educational status of pet owners were not statistically significant (P> 0.05). On antibiogram study 18 antibiotics were used for antimicrobial sensitivity test. Gram negative isolates were more sensitive against Chloramphenicol, Cephalexin, Gentamycin and Kanamycin. On the other hand gram positive isolates were more sensitive against Azithromycin, Levofloxacin, Colistin and Gentamycin. The isolates were highly resistant against Amoxycillin, Bacitracin, Penicillin and Vancomycin.

Keywords:- *Zoonotic Discease, Zoonotic Bacterial Pathogens, Statistically Study, Antibiogram Study.*

I. INTRODUCTION

Animal domestication has spread from long ago, the number of households keeping animals has increased and the relationship between human and animals has become closer under the life environment in contemporary society where technology has developed [1]. Our cave dwelling ancestors used dogs as the co-partner in hunting job. Subsequently, in all civilization dogs were used as guards, companions and hunters and in times of war. So, first pet animal of man was dogs and the relationship between human and dogs began 12,000-15,000 years ago and with cats nearly 5,000 years ago [2].

Dogs and cats have significant benefits to our society like companionship, play with children, guard the house and from any adverse condition alert the owner, used as gift to special one and economic purpose [3]. In many households contributing to the physical, social and mental development of children and the well-being of their owners, they act as important companion [4] [5].

Now-a-days, rabbits are the popular pets, coming third after dogs and cats and also still play an important role in the industrial sector and as laboratory animals [6]. Rabbits make excellent pets because they are clean, docile and calm by nature [7]. They are extremely delicate animals and are prone to many bacterial and fungal diseases if proper care is not taken and can also result in rabbit malnutrition, growth retardation, feed remuneration reduction and even death [8].

Pet animals kept for pleasures and companionship are usually domesticated and selectively bred for coexistence with human beings, besides their value as pets, pets serve utilitarian purposes protecting homes and property, destroying vermin and providing means of transport. They have been sharing our environment and have gained a major status as “pets” in our modern, very urbanized society. In the middle of the 20th century, they are more and more considered as “family members” within households; not to mention sometimes as substitutes for children [9].

Diseases and infections those are naturally transmitted between vertebrate animals and man are called zoonoses [10]. There are approximately 1415 pathogens known to affect humans of which about 61% of all human pathogens are zoonotic [11]. Household pets (dog, cat & rabbit), defined here as any animals kept within households by people for company, enjoyment, work or psychological support, can be colonized or infected with a wide variety of bacteria and fungi pathogenic to animals and people. Pet-associated bacterial and fungal zoonoses represent a relatively neglected area compared with food borne zoonoses [12].

In our country dogs, cats and rabbits are often purchased as a pet and these house-hold pets appear to be an important source of zoonotic diseases. Consequent to their popularity pet owners become increasingly knowledgeable and are willing to pay for advanced treatment and diagnostics [13]. Together with the increasing concern in the private, scientific and industrial sector to keep the animal healthy also the need regarding veterinarian expertise increased. Therefore, control of zoonotic diseases is even more important due to the increasing number of immunocompromised people but the distribution of pets around the world and their differences modify their role in zoonotic disease transmission. The risk of pathogen transmission from pet to the owner is relatively small, when simple precautions are taken. Therefore, the role of veterinarians is essential since they have to provide pet owners with accurate information [14]. Continuous investigative research provides new insights in clinical patterns, symptoms, etiologies and pathogenesis of different zoonotic infections/ diseases having public health significance to improve our knowledge of understanding.

II. METARIALS AND METHODS

The present study was carried out of pet animals (dog, cat and rabbit) under the sadar of Dinajpur district and samples (oral swab, skin scrapping, nasal swab, rectal swab, feaces and urine) were taken in the bacteriology laboratory of the department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh for the identification of bacteria & fungus by different microbiological methods.

The study was directed during the period from July 2016 to June 2017.

A. Plan of the experiment work at a glance

All of those samples were collected from different areas of Dinajpur sadar with a thermo flask containing ice in suitable diluent viz peptone water maintaining aseptic condition. Then all of the samples were transferred to the microbiological laboratory of department of Microbiology, HSTU, Dinajpur, Bangladesh. Appropriate amount of samples were primarily inoculated into Nutrient agar and Plate count agar for determining the density of bacterial profiles in pet animals that they carry and obtained their total viable count (TVC). Subsequently Nutrient agar, Blood agar, EMB agar, SS agar, MacConkey agar, Cetrimide agar and Staphylococcus agar NO. 110 base were employed and specific biochemical tests were done for isolation and identification of bacteria. At last performed antibiotic sensitivity test with the pure isolated bacteria.

B. Methods

- *Questionnaire*

A structured pre-coded questionnaire focusing on the information of pet animals and pet owners related to the transmission of zoonotic infection was written in English and converted to Bengali whenever needed during data collection. Socio-demographic variables of the study population such as age, sex, breed, body weight, housing system, hygienic condition, vaccination and education level of pets owner. Owner of pets in the study were interviewed by direct contact, visited their home or a few times by phone.

- *Collection and transportation of Samples*

A total of 404 samples (oral swab, nasal swab, skin scrapping, rectal swab, feaces, and urine) were collected from pet animals (dog, cat, rabbit) by sterile cotton buds and took into sterile tube containing with 1% peptone water. Each sample was marked properly with date, time and sample number then kept in an insulated ice box. After collection of those samples in a tube closed the cap and taken to the laboratory for microbiological investigation.

All samples were brought to the laboratory within half an hour of collection and subjected to bacteriological examination. Samples were kept under refrigeration at 4^0-7^0C until study.

- *Sampling and Processing of Samples*

Proper care was taken during the sampling procedure to prevent contamination of sample. The samples tubes were completely tied at the time of sampling that prevent contamination. After came to the laboratory the sample tubes were shake to mix with 1% peptone water. After that 9ml PBS (Phosphate Buffer Solution) was taken each test tube for serial dilution. Then 1ml sample was taken from each sample for ten-fold serial dilution (10^{-1} to 10^{-10}) and 50 µl samples were seeded on nutrient agar and plate count agar using spread plate method. The plating was done in the laminar flow to maintain aseptic conditions and the medium were then incubated at 37^0C for 24 h.

- *Microbial assessment of the collected samples*

Samples were collected and each of the samples was diluted with distilled water as 10^{-1} to 10^{-10}. Then 50µl samples were taken and spread in plate count agar(PCA) plate following the spread-plate method and incubated at 37ºC for 24 h. The number of organisms per ml or per gram of original culture was calculated by multiplying the number of colonies counted by the dilution factor: Number of cells per ml or per gram = number of colonies × Dilution factor/Volume of dilution.

➢ *Identification of the bacterial genera*

The bacterial isolates were transferred to sterilized plates for purification and identification. The grown bacteria were smear on a slide, stained with gram's stain solution to detect bacterial structures, examined under microscope (100X) and identified on the basis of their colony morphology then confirmed by biochemical test.

C. Antibiotic Sensitivity Test

The antibiotic resistance was determined by Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (Difco), according to the recommendations of National Committee for Clinical Laboratory Standards [15]. After overnight incubation at 37 °C, the diameter in millimeters of the zones of inhibition around each of the antimicrobial discs was recorded and categorized as resistant or sensitive in accordance with company recommendations. All isolates were tested for sensitivities to 18 of routine and practical antibiotics.

D. Statistical Analysis

Data were analyzed using SPSS for Windows (version 21.0). Prevalence of bacterial isolates was expressed in simple descriptive statistics such as means and standard deviation. For CFU/gm values, one-way ANOVA test and the detection of significant differences between ($p \leq 0.05$) socio demographic variable and zoonotic pathogens Chi-square test (χ^2) was done.

III. RESULTS

Socio demographic variable of study population with (dog, cat and rabbit) are as follows in TABLE 1 (A); 1 (B); 1 (C).

Socio demographic Profile		Frequency	Percentage (%)
Age	Young (<6 months)	5	10
	Adult (7-20 months)	15	30
	Old (>21months)	30	60
Sex	Male	24	48
	Female	26	52
Breed	Indigenous	23	46
	Exotic	27	54
Body wt.	<7 kg	5	10
	8-17 kg	17	34
	>18 kg	28	56
Housing System	Poor	20	40
	Good	21	42
	Excellent	9	18
Diet	Ready Feed	0	0
	Raw Food	38	76
	Both	12	24
Hygienic Condition	Poor	15	30
	Good	28	56
	Excellent	7	14
Vaccination	Yes	27	54
	No	23	46
Education level of pets owner	Under Graduate	20	40
	Graduate	12	24
	Post Graduate	18	36
Total		50	100

Table 1 (A):- Socio demographic variable of study population (in case of dog)

ISSN No:-2456-2165

Socio demographic Profile		Frequency	Percentage (%)
Age	Young (<6 months)	2	20
	Adult (7-12 months)	4	40
	Old (>13months)	4	40
Sex	Male	2	20
	Female	8	80
Breed	Indigenous	10	100
	Exotic	0	0
Body wt.	<1 kg	2	20
	1-3 kg	4	40
	>3 kg	4	40
Housing System	Poor	8	80
	Good	2	20
	Excellent	0	0
Diet	Ready Feed	0	0
	Raw Food	10	100
	Both	0	0
Hygienic Condition	Poor	8	80
	Good	2	20
	Excellent	0	0
Vaccination	Yes	0	0
	No	10	100
Education level of pets owner	Under Graduate	2	20
	Graduate	8	80
	Post Graduate	0	0
Total		10	100

Table 1 (B): Socio demographic variable of study population (in case of cat)

Socio demographic Profile		Frequency	Percentage (%)
Age	Young (<6 months)	2	10.53
	Adult (7-30 months)	10	52.63
	Old (>31months)	7	36.84
Sex	Male	11	57.89
	Female	8	42.11
Breed	Indigenous	19	100
	Exotic	0	0
Body wt.	<1 kg	2	10.53
	1-2 kg	11	57.89
	>2 kg	6	31.58
Housing System	Poor	4	21.05
	Good	15	78.95
	Excellent	0	0
Diet	Ready Feed	0	0
	Raw Food	7	36.84
	Both	12	63.16
Hygienic Condition	Poor	4	21.05
	Good	15	78.95
	Excellent	0	0
Education level of pets owner	Under Graduate	5	26.31
	Graduate	2	10.53
	Post Graduate	12	63.16
Total		19	100

Table 1 (C): Socio demographic variable of study population (in case of rabbit)

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A. Prevalence and Identification of Zoonotic Bacterial Pathogen

➢ *Results of Total Bacterial Count (TBC)*

TBC expressed as CFU/g (Colony Forming Unit per gram) of different samples from household pet animals were shown in TABLE 2. In case of dogs the average colony counts were 10.14±.76 for oral swab, 10.16±.78 for skin scrapping, 10.14±.75 for nasal swab and 10.13±.80 for rectal swab and 10.35±.54 for faces. In case of cats the average colony counts were 10.50±.04 for oral swab, 10.57±.057 for skin scrapping, 10.55±.11 for nasal swab and 10.63±.15 for rectal swab. In case of rabbits the average colony counts were 10.42±.23 for oral swab, 10.41±.242 for skin scrapping, 10.46±.28 for nasal swab, 10.47±.26 for rectal swab and 10.45±.29 for faces and 10.44±.25 for urine.

		Species		
		Dog	**Cat**	**Rabbit**
TBC (mean± SD) CFU/g	Oral swab	10.14±.76	10.50±.04	10.42± .23
	Skin scrapping	10.16±.78	10.57±.06	10.41±.242
	Nasal secretion	10.14±.75	10.56±.11	10.46±.28
	Rectal swab	10.13±.80	10.63±.26	10.47±.26
	Feaces	10.35±.54	ND	10.45±.29
	Urine	ND	ND	10.44±.25
P- Value		0.51	0.10	0.081

Table 2:- TBC of potentially zoonotic bacterial pathogen in dog, cat, and rabbit.

[All values are converted into logarithms 10; All counts are expressed in colony forming units (cfu); ND= Not Done; SD= Standard Division.]

Figure 1. Summarized the prevalence of zoonotic bacterial pathogen in pet dogs, cat & rabbit. In dog the overall prevalence of *E.coli; Klebsiella* spp*; Salmonella* spp*; Proteus* spp*; Pseudomonas* spp*; Staphylococcus* spp *and Streptococcus* spp were 15.2%; 10.8%; 12%; 8%; 8.8%; 12.8% and 10.4% respectively. In cat the overall prevalence of *E.coli; Klebsiella* spp*; Salmonella* spp*; Proteus* spp*; Pseudomonas* spp*; Staphylococcus* spp *and Streptococcus* spp were 17.5%; 10%; 12.5%; 10%; 10%; 15% and 15% respectively. And in rabbit the overall prevalence of *E.coli; Klebsiella* spp*; Salmonella* spp*; Proteus* spp*; Pseudomonas* spp*; Staphylococcus* spp *and Streptococcus* spp were 12.28%; 7.89%; 11%; 5%; 2%; 6.14% and 6.14% respectively.

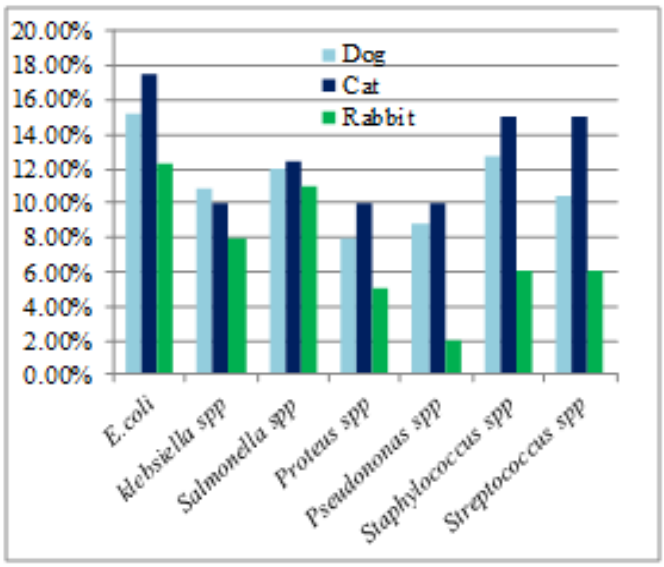

Fig 1:- Prevalence of potentially bacterial zoonotic pathogens in Dog, Cat & Rabbit.

B. Results of Cultural Examination

The cultural characteristics of *E. coli, Klebsiella spp, Salmonella* spp, *Proteus spp, Pseudomonas spp, Staphylococcus spp*, and *Streptococcus spp* on various media are presented in Table 3.

S/N	Name of bacteria	Staining charact-eristic	Name of media	Colony characteristics
01	*E. coli*	Gram negative large rod shaped pink colour.	NA	Large, mucoid, white colony.
			MaC	Produce large mucoid rose pink colony
			EMB agar	Transmitted light blue-black center with a narrow, clear edge. Blue-green metallic sheen with reflected light.
			BGA	Yellow-green colony.
02	*Klebsiella* spp	Gram negative rod shaped pink colour.	NA	Large colony.
			MaC	Large, red, mucoid
			EMB agar	Mucoid, no metallic sheen. With transmitted light, gray-brown centers and pink color with clear edges.
03	*Salmonella* spp.	Gram negative small rod shaped pink colour.	NA	Smooth. Opaque, translucent colonies.
			SS Agar	Opaque, smooth, round with black centered colonies.
			MaC	Small, white, translucent dew drop like colonies.
			BGA	Good growth red and pink white colonies.
04	*Proteus spp*	Gram negative small rod shaped.	NA	Circular, smooth, entire, opaque with white color colonies.
			MaC	Colourless and transparent colonies.
			BGA	Colonies of a pale pink color, transparent and surrounded by a brilliant red halo.
05	*Pseudomonas spp*	Gram negative small rod shaped pink colour.	NA	Large, smooth, low convex and greenish pigment with fruity odor.
			MaC	Pale colour flat non lactose fermenting colonies
			CA	Colonies are greenish in color.
			BA	β-hemolytic colonies.
06	*Staphylococcus spp.*	Gram positive cluster liked violet colour.	NA	Black colour/ non-colour smooth, glistening colonies.
			NB	Uniform turbidity.
			MSA	Yellow colonies.
			SA No.110	Yellow colonies.
			BA	β-hemolytic colonies.
07	*Streptococcus spp*	Gram positive short chain shaped violet colour.	NA	Uniform turbidity.
			NB	Moderate growth.
			MSA	Pink colony
			BA	Small, dry colony surrounded by β-hemolysis.

Table 3:- The result of cultural characteristics of the bacteria which are isolated from different samples of pet animals (dog, cat and rabbit).

[Where; NA = Nutrient Agar; NB = Nutrient Broth; MaC = Mac-Conkey's Agar; EMB = Eosin Methylene Blue; BGA = Brilliant Green Agar; SS = Salmonella-Shegilla Agar; CA = Cetrimide agar; BA = Blood Agar; MSA = Mannitol Salt Agar; SA No. 110 = *Staphylococcus* Agar No. 110]

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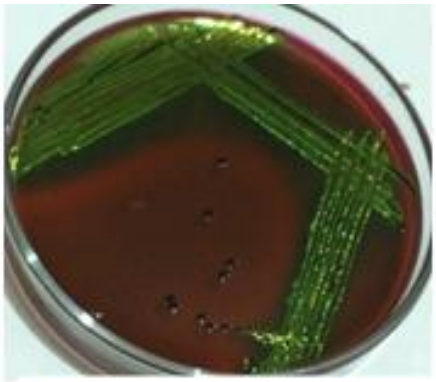

Fig 2:- *E.coli* on Eosin Methylene Blue agar

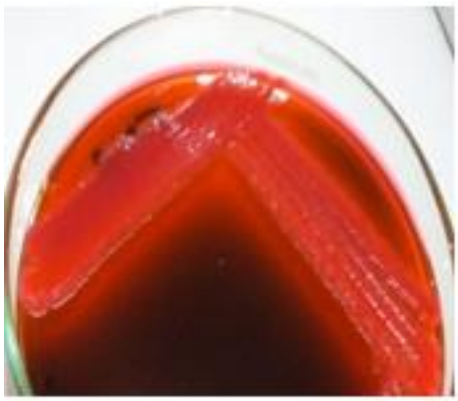

Fig 3:- *klebsiella* spp on Eosin Methylene Blue agar

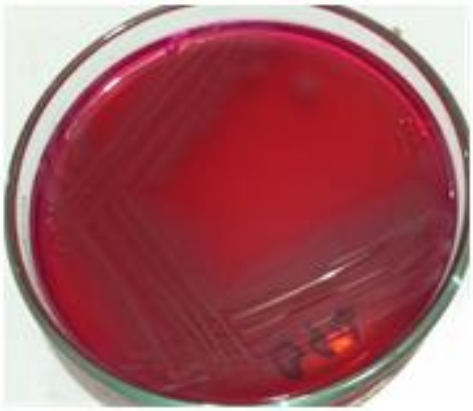

Fig 4:- *Salmonella spp* on Brilliant Green Agar

Fig 5:- *Proteus* spp on Mac-Conkey Agar

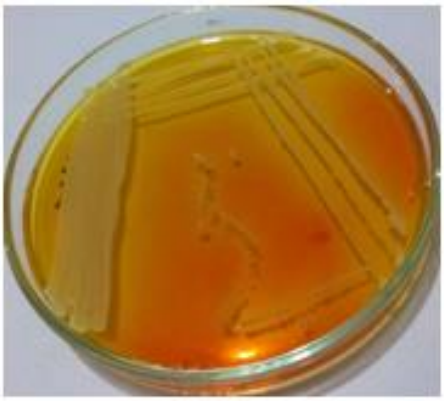

Fig 6:- *Staphylococcus spp* on Mannitol Salt

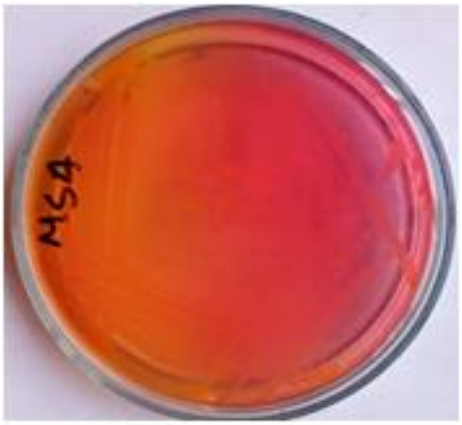

Fig 7:- *Streptococcus spp* on Mannitol Salt Agar

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Species	Sex	Age	TBC (mean ± SD) CFU/g					
			OS	SS	NS	RS	F	Urine
Dog (n=50)	Male (N=24)	Young (<6 M)	9.05 ±2.22	9.66 ±1.15	9.16 ±2.06	8.69 ±2.81	9.21 ±2.07	ND
		Adult (7-20 M)	10.33 ±0.36	10.13 ±0.79	10.22 ±0.36	10.27±0.35	10.33 ±0.32	ND
		Old (>21 M)	9.99 ±0.94	10.03 ±1.17	9.93 ±0.62	9.88 ±0.95	10.29 ±0.99	ND
		P- Value	0.187	0.818	0.316	0.148	0.065	ND
	Female(N=26)	Young (<6 M)	9.76 ±0.49	9.83 ±0.48	10.07 ±0.46	9.998 ±0.43	10.31 ±0.25	ND
		Adult (7-20 M)	10.52 ±0.25	10.56 ±0.22	10.51 ±0.18	10.47±0.28	10.61 ±0.12	ND
		Old (>21 M)	10.13 ±0.74	10.18 ±0.67	10.17 ±0.75	10.21±0.63	10.41 ±0.46	ND
		P- Value	0.25	0.201	0.495	0.465	0.469	ND
Cat (n=10)	Male (N=2)	Young (<6 M)	NB	NB	NB	NB	ND	ND
		Adult (7-12 M)	10.53 ±0.08	10.53 ±0.08	10.75 ±0.02	10.74±0.01	ND	ND
		Old (>13 M)	NB	NB	NB	NB	ND	ND
		P- Value	0.003	0.003	0.001	0.00	ND	ND
	Female(N=8)	Young (<6 M)	NB	NB	NB	NB	ND	ND
		Adult (7-12 M)	10.51 ±0.05	10.52 ±0.05	10.48 ±0.02	10.45±0.02	ND	ND
		Old (>13 M)	NB	NB	NB	NB	ND	ND
		P- Value	0.000	0.000	0.000	0.000	ND	ND
Rabbit(n=19)	Male (N=11)	Young (<6 M)	10.59 ±0.17	10.64 ±0.22	10.69 ±0.1	10.52±0.39	10.72 ±0.06	10.26±0.18
		Adult (7-30 M)	10.42 ±0.17	10.43 ±0.18	10.42 ±0.31	10.44±0.26	10.42 ±0.29	10.51±0.12
		Old (>31 M)	10.39 ±0.16	10.49 ±0.02	10.53 ±0.18	10.52±0.25	10.22 ±0.22	10.51±0.17
		P- Value	0.36	0.33	0.42	0.85	0.17	0.098
	Female(N=8)	Young (<6 M)	NB	NB	NB	NB	NB	NB
		Adult (7-30 M)	10.53 ±0.37	10.48 ±0.39	10.55 ±0.37	10.59 ±0.34	10.61 ±0.35	10.52±0.39
		Old (>31 M)	NB	NB	NB	NB	NB	NB
		P- Value	0.000	0.000	0.000	0.000	0.000	0.000

Table 4:- Species; sex & age wise distribution of total viable count (TVC) of zoonotic bacterial pathogen from dog; cat; rabbit.

[WHERE; All values are converted into logarithms 10; All counts are expressed in colony forming units (CFU); M = Months; ND= Not Done; NB= Nobody; SD = Standard Division; SS=Skin Scrapping; NS = Nasal Secretion; RS=Rectal Swab; OS = Oral Swab; F = Feaces]

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Breed	Samples	TBC (mean+ SD) CFU/g	P- Value
Local/ Indigenous (n=23)	Oral swab	9.99 ± 0.77	.0001
	Skin scrapping	9.99 ± 0.86	
	Nasal secretion	10.14 ± 0.83	
	Rectal swab	2.33 ± 1.57	
	Feaces	10.30 ± 0.65	
Exotic (n=27)	Oral swab	10.25 ± 0.75	.0001
	Skin scrapping	10.20 ± 0.81	
	Nasal secretion	10.14 ± 0.71	
	Rectal swab	10.15 ± 0.703	
	Feaces	10.36 ± 0.47	

Table 5:- Breed wise distribution of TBC of zoonotic bacterial pathogen from pet dogs.

[Where, All values are converted into logarithms 10; All counts are expressed in colony forming units (CFU); 0.0001 means statistically highly significant.]

Serial Test Parameters		1	2	3	4	5	6	7
SF	Lac	AG	AG	-	-	-	A	-
	Dex	AG	AG	AG	AG	-	A	A
	Suc	A	AG	A	-	-	A	A
Oxidase		-	-	-	-	+	-	-
Catalase		+	+	+	+	+	+	-
Indole		+	-	-	+	-	-	-
MR Reaction		+	-	+	+	-	+	-
VP Reaction		-	+	-	+	-	-	+
SC		-	+	+	+	+	-	-
Ornithine		-	+	+	+	+	-	-
TSI		YY	YY	YR	YR	RR	YR	YR
MIU		+	-	+	+	+	+	+
Selenite		+	+	+	+	+	-	-
Results		*E.coli*	*Klebsiella spp*	*Salmonella Spp*	*Proteus spp*	*Pseudomonas spp*	*Staphylococcus spp*	*Streptococcus Spp*

Table 6:- Results of Biochemical Tests of isolated bacteria

[Where; A= Acid, G= Gas, + = positive, - = negative, YY= Yellow-yellow; YR= Yellow-Red, SF= Sugar Fermentation, Lac= Lactose, Dex= Dextrose, Suc= Sucrose, MR= Methyl Red, VP= Voges-Proskaur, SC= Simmons Citrate, TSI= Triple Sugar Iron, MIO= Motility Indole Urease]

C. *Antibiotic Sensitivity Test*

On antibiogram study 18 antibiotics were used for antimicrobial sensitivity test. Gram negative isolates were more sensitive to Chloramphenicol, Cephalexin, Gentamycin and Kanamycin. On the other hand gram positive isolates were more sensitive to Azithromycin, Colistin, Gentamycin and Levofloxacin. On the other hand Azithomycin; Chloramphenicol were intermediate for gram negative bacteria. The all isolates were highly resistant to Amoxycillin, Bacitracin, Penicillin and Vancomycin. Gram positive isolates were more sensitive to Azithromycin, Colistin, Gentamycin and Levofloxacin.

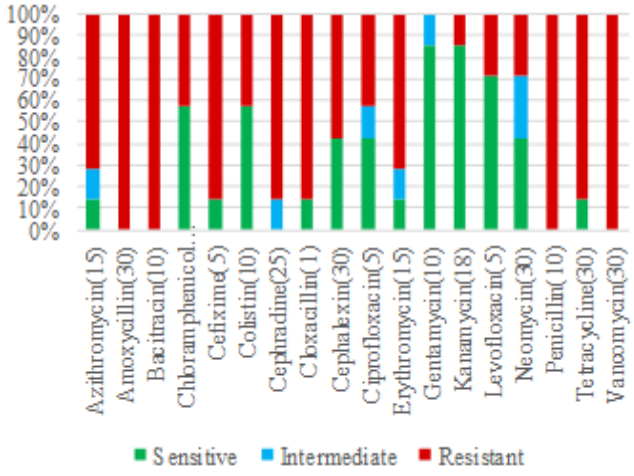

Fig 8:- Column diagram presenting antibiotic sensitivity test of isolated Bacteria

IV. DISCUSSION

The results of the current study revealed that the prevalence of different bacterial pathogen in relation to socio-demographic variable in case of housing system and hygienic condition were statistically significant (P<0.05) whereas in case of age, sex, body weight, diet and the educational quality of pet owners were not statistically significant(P>0.05).

The results of total bacterial count (TBC) are not statistically significant (P>0.05) among the different samples and pet animals showed in TABLE III. The TBC in oral swab, skin scraping, nasal swab, rectal swab and feces samples in case of dog were 10.14±.76 CFU/gm; 10.16±.78 CFU/gm; 10.14±.75 CFU/gm; 10.13±.80 CFU/gm; 10.35±.54 CFU/gm respectively. In case of cat oral swab, skin scarping, nasal swab and rectal swab samples were 10.50±.04 CFU/gm; 10.57±.057 CFU/gm; 10.55±.11CFU/gm and 10.63±.15CFU/gm respectively. In case of rabbit oral swab, skin scarping, nasal swab, rectal swab, feces and samples were 10.42±.23 CFU/gm; 10.41±.24 CFU/gm; 10.45 ±.28 CFU/gm; 10.47±.26 CFU/gm; 10.45±.29 CFU/gm and 10.43±.25 CFU/gm respectively. Species, sex and age wise distribution of total viable count of zoonotic bacterial pathogen from pet animals showed in TABLE IV. This value analyzed by one way ANOVA test and these values are not statistically significant (P>0.05). Species and breed wise distribution of total viable count of zoonotic bacterial pathogen from household pet dogs showed in TABLE V and all the values are statistically significant (P<0.01). The overall prevalence of isolated bacteria in case of dog out of 250 samples were 15.2% *Escherichia coli;* 10.8% *Klebsiella* spp*;* 12% *Salmonella* spp*;* 8% *Proteus* spp*;* 8.8% *Pseudomonas* spp*;* 12.8% *Staphylococcus* spp *and* 10.4% *Streptococcus* spp respectively. The overall prevalence of isolated bacteria in case of cat out of 40 samples were 17.5% *Escherichia coli;* 10% *Klebsiella* spp*;* 12.5% *Salmonella* spp*;* 10% *Proteus* spp*;* 10% *Pseudomonas* spp*;* 15% *Staphylococcus* spp *and* 15% *Streptococcus* spp respectively. The overall prevalence of isolated bacteria in case of rabbit out of 114 samples were 12.28% *Escherichia coli;* 7.89% *Klebsiella* spp*;* 11% *Salmonella* spp*;* 5% *Proteus* spp*;* 2% *Pseudomonas* spp*;* 6.14% *Staphylococcus* spp *and* 6.14% *Streptococcus* spp respectively. Isolated *E. coli*, *Pseudomonas* spp, *Proteus* spp and *Staphylococcus* spp from dog and cat which commonly causes infectious diseases and transmitted from animal to human by direct contact [16]. Prevalence of *Salmonella* ranging from 0-9% and 0-4% in dogs and cats respectively and higher prevalence may be found in stray or shelter dogs/cats [17]. This organism can be transmitted directly or indirectly by the fecal-oral rout and develops the symptoms of gastroenteritis in human [18]. In a study conducted by Hashemi, reported that the prevalence rate of *Salmonella* were 18 and 22 % in cat and respectively which agree with the current study [19]. Another one reported that the prevalence of Salmonellosis in dog and cat were 23.15% and 13.05% respectively in Dhaka, Bangladesh [20]. *E.coli* are the part of the normal intestinal micro-flora, but they can cause gastroenteritis when local or systemic immunity does not work properly. Enteropathogenic strains of *E. coli* (EPEC) have been found in human patients and dogs that live in the same household [21]. Recent findings indicate that the transmission of diarrheagenic *E. coli* strains occurs between dogs and human. Asymptomatic dogs were identified as carrier of human pathogenic Shiga-toxin-producing (STEC) *E. coli* play an important role in outbreaks of STEC infections in human [22] [23]. Diarrheic dogs were identified as an important source of bacterial contamination of the environment in apartments of dog holders that might contribute to the spread and transmission of pathogenic *E. coli* strains [24]. *Staphylococcus* spp in a human source bacteria primarily in our anterior nares and on hands, cats and less commonly dogs may carry *S. aures* in their normal skin and mucosal bacterial flora [25]. But *S. pseudintermedius*is a dog or cat source bacteria found in cases of pyoderma, otitis and wound infections. This species is the most common cause of *Staphylococcus* infections in pets and also a major cause of dog bite infection in people. Belli reported that the prevalence rate of diluent bacterial isolates in rabbit were *Streptococcus* spp (22.8%) *Staphylococcus* spp (17.8%), *E.coli* (12.5%), *proteus* spp (5.7%) which supports the findings of the current study [26]. On the other hand, Martino and Luzi found that the prevalence of *Klebsiella* spp*; Pseudomonas* spp; *Staphylococcus* spp*; Streptococcus* spp and *Escherichia coli* were 9.4%, 15.6%, 6.2%, 3.1% and 3.1% respectively in rabbit. These findings are as agreed with the results of the current study [7]. Okumu isolated that *Escherichia coli* and *Staphylococcus aureus* were frequently isolated from conjuctival and nasopharyngeal swabs were 4.17% and 8.33% respectively which findings are also agreement with the results of the current study [27].

Martino described that samples came mainly from nose (swabs) with a prevalence of 37.5%, then from abscesses or abdominal cavity (28.1%) and from eyes (15.6%). *Pasteurella multocida* was the most isolated bacterium (21.9%) followed by *Pseudomonas aeruginosa* (15.6%) and *Klebsiella pneumonia* (9.4%). The other microorganisms were isolated in a low percentage. Many samples (28%) were negative probably due to sampling mistakes or to the poor significance of the collected sample (e.g, insufficient drawn material or sampling not in the correct site) or to antibiotic treatment of animals just before sampling. These data are in agreement with the high spreading of these types of pathologies in pet rabbits as reported by current study [7]. Dogs have been reported to be the carrier of *Salmonella* spp worldwide which have the potential to serve as sources of exposure or infection for humans [28]. It was reported that the intestinal carriage of salmonellae by dogs is more common than the prevalence of clinical disease. The frequency of faecal isolation of *Salmonella spp.* from clinically healthy dogs was reported to be between 0.0% and 43.0% [28] [29]. The current study found 12% positive result for *Salmonella* spp from dog.

ISSN No:-2456-2165

On antibiogram study 18 antibiotics were used against 7 isolated bacteria for antimicrobial sensitivity test. Cephalexin and kanamycin were 100% sensitive for all gram negative isolates. Whereas Chloramphenicol was 100% sensitive to *E.coli; Klebsiella* spp; *Proteus* spp and *Pseudomonas* spp but 66.7% resistant to *Salmonella* spp. On the other hand ciprofloxacin was 100% sensitive for *Klebsiella* spp and *Proteus* spp but 100% resistant to *E. coli*; *Salmonella* spp and *Pseudomonas* spp. Again erythromycin was 100% sensitive to *Pseudomonas* spp but 66.7% resistant and 33.3% intermediate to *E.coli; Klebsiella* spp; *Proteus* spp and *Salmonella* spp. Gentamycin was 66.7% intermediate to *Salmonella* spp but100% sensitive to *E.coli; Klebsiella* spp; *Proteus* spp and *Pseudomonas* spp. Neomycin was 100% sensitive to *E.coli* and *Proteus* spp. Azithromycin, colistin, gentamycin and levofloxacin were 100% sensitive to *Staphylococcus* spp. Chloramphenicol, cephradine, ciprofloxacin, erythromycin, neomycin, kanamycin and cephalexin were 66.7-100% resistant to *Staphylococcus* spp. Amoxycillin, bacitracin, cefixime, cloxacillin, penicillin, tetracycline and vancomycin were 100% resistant to all isolates. According to Gerding, the most effective antibiotics for *Staphylococcus* spp. were bacitracin, gentamicin and tobramycin; while chloramphenicol and erythromycin for *Streptococcus* spp. Prado, showed that 80.7% of the isolates were gram positive cocci and gram positive bacilli, and those species were sensitive to gentamicin, ciprofloxacin, chloramphenicol and tobramycin. This result is agreed with the findings of the current study [30] [31]. *Staphylococcus* species isolated from dogs with pyoderma were found to be resistant to streptomycin, kanamycin, neomycin and erythromycin (28%), to clindamycin (22%) and to gentamicin and enrofloxacin [32]. Keskin reported that 82.5% of the bacteria isolated from the dogs with otitis externa were resistant to enrofloxacin, 65.5% to cephalosporins, 44.4% to gentamicin and tetracycline, 34.9% to spiramycin, 26.9 to ampicillin, while 20.6% were resistant to lincomycin [33]. Sarierler reported that bacteria isolated from dogs with otitis externa were resistant to oxytetracycline (100%), ciprofloxacin (100%), kanamycin (87.5%), penicillin G (72.5%), erythromycin (57.5%), gentamicin (55%), ampicillin (50%) and cefoperazone (50.0%). Keskin was reported that *Staphylococcus* species were highly resistant (63.1%) to ampicillin. Hariharan reported that most isolates were susceptible to gentamycin but resistant to ampicillin, penicillin. Schick reported that most isolates were susceptible to gentamycin (81%) and enrofloxacin (56%). Keskin reported that most bacterial strains from dogs were sensitive to enrofloxacin, gentamycin, and lincomycin, but resistant to tetracycline and ampicillin. Martin reported that most isolates were susceptible to tobramycin (100%), marbofloxacin (90%), ceftazidime (90%), gentamycin (68%), and enrofloxacin (42%). All finding are nearly agreement with the results of the current study [33] [34] [35] [36] [37].

V. CONCLUSIONS

Since the isolation bacteria can be potentially pathogenic to people, this study comes to demonstrate that there is on the contact of human to household pets, despite the absence of the clinical signs and symptoms from the zoonotic bacterial infection. Also, children may snuggle with pets can increase the risk zoonotic disease transmission. Hence the owner of the pets should have correct knowledge about zoonoses for their prevention and should take precautionary measures, improve personal hygienic to reduce the risk contact with pathogenic bacteria.

ACKNOWLEDGMENT

Authors are very thankful to all staff of Department of Microbiology, Faculty of Veterinary and Animal Science (FVAS), Hajee Mohammad Danesh Science and Technology University (HSTU) for their cooperation and supporting laboratory works.

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