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Phenotypic and Genotypic Characteristics of Antimicrobial Resistance of Gram-negative Bacteria Isolated from Pet Animal

Marwa M. Fathi[1], Ahmed Samir[2], Sherif Marouf[2], Aisha R. Ali[1], Khaled Al-Amry[2*]

[1]Animal Health Research Institute, Agriculture Research Center, Dokki, P.O. Box 246, Giza 12618, Egypt.
[2]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.

***Correspondence**
Khaled Al-Amry
Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, 12211, Egypt.
E-mail address: kelamry@darelfarouk.com.eg

Abstract

Most animal feeds are set from protein-rich raw materials. These protein constituents may possess various hazards, particularly highly drug-resistant pathogens, causing a bad impact not only on the pet's health, but also on their owners. In the current study, a total of 2100 pet food and 100 pets' fecal swabs were collected and bacteriologically examined from 2017 to 2020. It was revealed that the percentage of Gram-negative bacteria isolated from pet food and fecal swabs was 49% and 56% respectively. *E. coli*, *Proteus* sp., and *K. pneumoniae* were the most isolated bacteria in percentages of 12.4%, 8.4%, and 4.9% respectively from Pet food and 25%, 7%, 12% respectively from pet fecal swabs. In addition, *Enterobacter cloacae*, *P. aeruginosa*, *Aeromonas hydrophila*, *Citrobacter* sp., *P. fluorecens*, and *Y. enterocolitica* were isolated from pet food in an order of 3.8%, 3.5%, 3.2%, 2.6%, 2.6% and 2.1% respectively. *Salmonella* sp. isolated from pet food was 0.6% while it was 5% from pet fecal swabs. The most predominant *Salmonella* serotype isolated from pet food and pet fecal swabs was *S.* Typhimurium. Furthermore, *S.* Virchow, *S.* Anatum, *S.* Kentucky, *S.* Kedougou and *S.* Infantis were isolated serotypes from Pet food in percentages of 15.7%, 23.1%, 15.4%, 7.7%, and 7.7% respectively. While *S.* Nitra, *S.* Ibargi, *S.* Enteritidis and *S.* Boecker were isolated from pet fecal swabs at a percentage of 20% for each. On the other hand, O158 was the most predominant *E. coli* serogroup isolated from pet food and pet fecal swabs in percentages of 30.4% and 30.8% respectively followed by O157 in percentages of 21.7% and 26.9% respectively. O26 was isolated from pet food and pet fecal swabs in percentages of 13% and 7.7% for each. O119 was isolated from pet food and pet fecal swabs in percentages of 4.3% and 3.8% respectively. O86, O27, O44, O55, and O78 were isolated from pet food in the percentage of 4.3%, 8.7%, 4.3%, 4.3%, and 8.7%respectively. While O114, O111, and O125 were isolated serotypes from pet fecal swabs in percentages of 15.4%, 3.8%, and 11.5% respectively. This study revealed that the antimicrobial sensitivity test of 80% of *Salmonella*e were resistant to Cefotaxime and Colistin sulphate while 50%, 30, and 20% of isolates were resistant to Gentamicin, Tetracycline, and Cefepime respectively, while 40% of *Salmonella*e were resistant to Chloramphenicol, Enrofloxacin, and Amoxicillin-clavulanate. Also 60% of *Salmonella*e showed resistance to Trimethoprim sulfamethoxazole and Ciprofloxacin. Detection of Extended-spectrum ß-lactamase resistance genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) in Pets using Polymerase chain reaction (PCR) showed the presence of bla_{TEM} and bla_{SHV} genes in all tested isolates in 12 samples out of 12 (100%) and has shown that the ratio of bla_{CTX-M} is 5 out of 12samples (41.6 %). It could be concluded that ESBLs are widely present in pets' food and feces, which may be a potential reservoir of antimicrobial resistance, increasing the risk to the public and animals.

KEYWORDS
Pets, Antimicrobial resistance, ESBL, Gram negative bacteria, *Salmonella* sp., *E. coli*

INTRODUCTION

In recent years, the number of home animals, also known as pets, has grown tremendously. Dogs and cats have become popular pets in Egypt (Abdel-Moein and Samir, 2011). Increasingly, such animals require industrially produced food, most often referred to as pet food (Wojdat *et al*., 2004). The preparation and consumption of pet food should be safe for both animals, human and the environment (International Organization for Standardization, 2018). Pet food can pose many hazards due to the presence of biological, physical, or chemical agents in animal feed that may cause illness and injury to pets, without adequate production control measures (Kazimierska *et al*., 2021). Good microbiological quality of food is the main factor, in addition to nutritional value, in producing healthy and safe food (Chlebicz and Śliżewska, 2018). Numerous research reports have documented pet food quality problems and their impact on human and animal health. According to a recent study, pathogenic microorganisms (bacteria, fungi, and their toxins) constitute approximately 20% of all alerts submitted to the Rapid Alert System for Food and Feed (RASFF) system, and *Salmonella*, Listeria, and *E. coli* are among the most common to be reported. (RASFF, 2018; Pigłowski, 2019). Animal feed prepared from protein-rich raw materials is the most common source of this pathogen (Rönnqvist *et al*., 2018; Minh *et al*., 2020).

It has also been reported that processed pet foods contain

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other pathogens, such as Listeria, *Enterobacteriaceae*, and Campylobacter (van Bree *et al*., 2018; Hellgren *et al*., 2019). The microbiological quality and the high prevalence of antimicrobial-resistant *Enterobacteriaceae* in raw meat-based diets raise health concerns for both animals and humans. In 62.7% of the samples, antimicrobial-resistant bacteria were detected. A majority of these strains are resistant to third generation cephalosporins due to extended-spectrum β-lactamases (ESBLs), including CTX-M-1, which is prevalent in livestock, and CTX-M-15, the most common ESBL variant worldwide. (Nüesch-Inderbinen *et al*., 2019).

Extended Spectrum Beta Lactamase (ESBL) is an enzyme produced by Gram-negative bacteria *Enterobacteriaceae*, which can hydrolyze penicillin but also third-generation cephalosporin (Kristianingtyas *et al*., 2020). The spread of extended-spectrum β-lactamases (ESBLs) are a global public health issue. Most ESBL genes are mutant derivatives of the classical bla_{SHV} and bla_{TEM} β-lactamases, but a rapid increase in the prevalence of bla_{CTX-M} has been reported among *Enterobacteriaceae* over the past decade. These genes are capable of conferring resistance to third generation cephalosporins (e.g. ceftazidime and cefotaxime) and aztreonam, but not cephamycins (e.g. cefoxitin) and carbapenems (Memariani *et al*., 2015)

This study planned to throw light on the prevalence of microbial contamination of pet food fecal swabs via isolation of Gram-negative bacteria from pet food and fecal swabs, Evaluation of the antimicrobial susceptibility and multidrug resistance (MDR) profiles of bacterial species isolated from pet food and fecal swabs, and Detection of genes encoding the Extended-spectrum ß-lactamases (ESBLs) including bla_{TEM}, bla_{SHV}, and bla_{CTX-M} groups among the different Gram-negative isolates.

MATERIALS AND METHODS

Sample collection and preparation

The study comprised 2100 mixed canned and dried pet food samples, and 100 pets' fecal swabs of diseased and apparently healthy dogs and cats were submitted to the serology unit, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt from 2017 to 2020. The samples were soaked in peptone buffer saline Under complete aseptic conditions in a box with ice packs (at 4°C) and transferred to the laboratory. All procedures were approved by Animal Health Research Institute (AHRI) ethical Committee.

Bacteriological Examination

One gram from each sample was inoculated in a tube containing 9 ml of 1% buffered peptone water then incubated at 37°C for 18-24 h. From the first enrichment, 0.1 ml was incubated for 18-24 h at 42°C in 10 ml of Rappaport Vassiliadis (RV) broth (Oxoid), the incubated samples were inoculated onto MacConkey agar plates. Then developed colonies depended on macroscopic and microscopic appearance, were subcultured on appropriate differential media.

Expected colonies were streaked on specific media for *Salmonella*e, brilliant green (BG) agar and xylose-lysine-deoxycholate (XLD) agar (Oxoid), *P. aeruginosa*, Pseudomonas cetrimide agar medium (Oxoid), *E. coli* Eosin methylene blue (EMB agar) plates, then aerobically incubated at 37°C for 18-24 h. Suspected colonies were subjected to biochemical analysis as described by the International Organization for Standardization (2017) and Quinn *et al*. (2013).

Serological Confirmation

The biochemically identified *Salmonella* isolates were subjected to serological identification by monovalentantisera by slide agglutination test according to International Organization for Standardization (2014), part III. Diagnostic omnivalent A-67, polyvalent A-E, F-67 and monovalent *Salmonella* O and H (phase 1 and phase 2) antisera. (Denka Seiken co., LTD- Japan). *E. coli* strains were serogrouped by the usage of rapid diagnostic *E. coli* antisera Set 1 containing monovalent and polyvalent O antisera (Denka Seiken Company, LTD-Japan).

Antimicrobial susceptibility testing

Antimicrobial susceptibility profiles according to the International Organization for Standardization (2017) were determined by disc diffusion technique on Mueller Hinton agar according to the guidelines and interpretation criteria of the Clinical and Laboratory Standards Institute (CLSI, 2020). It was determined for ten antimicrobials discs (Oxoid, UK) (tetracycline (TE 30 µg), enrofoxacin (ENR 5 µg), sulphamethoxazole trimethoprim (SXT 25 µg), cefotaxime (CTX 30 µg), cefepime (FEP 30 µg), ciprofloxacin (CIP 5 µg), gentamicin (CN 10 µg), chloramphenicol (C 30 µg), amoxicillin/clavulanic acid (AMC 30 µg), and colistin sulphate (CT 10 µg) represented to Eight antimicrobial groups have been used. The diameters of growth inhibition for different antibiotics were interpreted according to the table established by CLSI (2020).

PCR screening for resistance genes

The DNA extraction from 12 pet food and fecal samples has been completed through the usage of the QIA-amp DNA Mini kit (Qiagen, GmbH, Germany). Oligonucleotide primers have specific sequences (Metabion, Germany), targeted genes and their amplified fragment sizes are shown in Table 1. These primers were applied in a 25ul reaction including 12.5 ul of Emerald Amp Max PCR Mastermix (Takara, Japan), 1 ul of separate primer of 20 picomole concentrations, 5.5 ul of water and 6 ul of the template DNA. The cycling conditions for detection of bla_{CTX}, bla_{TEM}, and bla_{SHV} were as follows: initial denaturation at 95°C for 5 min, 35 cycles of 94°C for 30 sec, 54°C for 40 sec and 72°C for 45 sec, and a final elongation at 72°C for 10 min for bla_{CTX} and 72 for 7min for bla_{TEM}, and bla_{SHV}. In a Biometra thermal cycler, the PCR outcomes

Table 1. Oligonucleotide primers sequences (Source: Midland Certified Reagent Company oilgos (USA).

Gene	Primer Sequence 5'-3'	Amplified product (bp)	Reference
bla_{TEM}	ATCAGCAATAAACCAGC	516	Colom *et al.*. (2003)
	CCCCGAAGAACGTTTTC		
bla_{SHV}	AGGATTGACTGCCTTTTTG	392	
	ATTTGCTGATTTCGCTCG		
bla_{CTX-M}	ATG TGC AGY ACC AGT AAR GTK ATG GC	593	Archambault *et al.* (2006)
	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG		

had been separated by way of electrophoresis in 1.5% agarose gel (ABgene, Germany). One hundred base pair and 100¬600 base pair deoxyribonucleic-acid ladders (Qiagen, USA) decide the fragment sizes were used. The gel pictured with the aid of a documentation device and the records stored via a computer software program.

Statistical analysis

Data analysis was conducted by PASW Statistics, Version 18.0 software (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher's Exact tests were employed to test the correlation between bacterial isolates and specimen type. A P-value smaller than 0.05 was regarded as significant.

RESULTS

Bacterial isolation and identification

Out of 2100 pet food samples and 100 pets' fecal swabs, 1029 (49%) and 56 (56%) respectively were positive to isolated Gram-negative bacteria which biochemically identified as *E. coli*, *Proteus* sp. and *Klebsiella pneumoniae* were the most isolated bacteria in percentage of 12.4%, 8.4% and 4.9% respectively from Pet food and 25%, 7% and 12% respectively from pet fecal swabs. *Enterobacter cloacae* (3.8%), *P. aeruginosa* (3.5%), *Aeromonas hydrophila* (3.2%), *Citrobacter* sp. (2.6%), *P. fluorescens* (2.6%), *Y. enterocolitica* (2.1%) were isolated from Pet food. *Salmonella*e were isolated from pet food in percentage of 0.6% while it was 5% from pet fecal swabs as shown in Table 2.

Serological identification of Salmonella sp. and E. coli

As shown in Tables 3 and 4, the most predominant *Salmonella* serotype isolated from pet food and pet fecal swabs were *S.* Typhimurium. While *S.* Virchow, *S.* Anatum, *S.* Kentucky, *S.* Kedougou and *S.* Infantis were isolated by serotyping from Pet food in percentage of 15.7%, 23.1%, 15.4%, 7.7% and 7.7% respectively. While *S.* Nitra, *S.* Ibargi, *S.* Enteritidis and *S.* Boecker were isolated by serotyping from pet fecal swabs in percentage of 20% for each.

In this study, O158 was the most predominant *E. coli* serogroup isolated from pet food and pet fecal swabs in percentage

Table 2. Prevalence of bacteria isolated from pets' animal food (N= 2100) and pets' fecal swabs (N= 100).

Type of isolated gm -ve bacteria	No. of isolates/2100 food samples (%)	No. of isolates/100 fecal swabs (%)	*p*-value
E. coli	261 (12.4) [a]	25 (25.0) [a]	0.451
Proteus sp	176 (8.4) [b*]	7 (7.0) [b]	0.034*
Klebsiella pneumoniae.	104 (4.9) [c]	12 (12.0) [b]	0.261
Enterobacter cloacae	79 (3.8) [cd]	7 (7.0) [b]	0.872
Pseudomonas aeruginosa	73 (3.5) [d]	0	
Aeromonas hydrophila	67 (3.2) [de]	0	
Citrobacter sp.	55 (2.6) [ef]	0	
Pseudomonas fluorescens	54 (2.6) [ef]	0	
Yersinia enterocolitica	44 (2.1) [f]	0	
Salmonella sp	13 (0.6) [g]	5 (5.0) [b*]	0.001*
Non identified m.o	103 (4.9) [c]	0	
p-value	< 0.0001	< 0.0001	

[a,b,c] Different superscripts in the same column indicate significance at p< 0.05. * Indicate significance in the same row at p< 0.05.

Table 3. Prevalence of *Salmonella* serovars from positive pets' animal food (n= 13) and pets' fecal swabs (n= 5).

Salmonella serovars	*Antigenic structure	Pet food (n= 13)		Fecal swabs of pet (n= 5)		
		No.	%	No.	%	*p*- value (*FET*)
S. Typhimurium	1,4,[5],12: i:1,2	4	30.8	1	20	1
S. Virchow	6,7,14: r:1,2	2	15.4	0	0	
S. Anatum	3,{10}{15}{15,34}:e,h:1,6 [z_{64}]	3	23.1	0	0	
S. Kentucky	8,20: i :z_6	2	15.4	0	0	
S. Kedougou	1,13,23 :i :l,w	1	7.7	0	0	
S. Infantis	6,7,14:r :1,5	1	7.7	0	0	
S. Nitra	2,12:g,m	0	0	1	20	
S. Ibargi	21 :y :1,2	0	0	1	20	
S. Enteritidis	1,9,12:g,m	0	0	1	20	
S. Boecker	[1],6,14,[25] :l,v :1,7	0	0	1	20	
p- value (*FET*)			0.709		1	

*The antigenic structures of the isolated *Salmonella* serovars which cover groups B, C, D, F, G, H, K and L according to modified Kauffman-White scheme (2007)

of 30.4% and 30.8% respectively followed by O157 in percentage of 21.7% and 26.9% respectively. Also, O26 was isolated from pet food and pet fecal swabs in percentage of 13% and 7.7% for each. In addition, O119 was isolated from pet food and pet fecal swabs in percentage of 4.3% and 3.8% for each. O86, O27, O44, O55 and O78 were isolated from pet food in percentage of 4.3%, 8.7%, 4.3%, 4.3% and 8.7% respectively. While O114, O111 and O125 were isolated from pet fecal swabs in percentage of 15.4%, 3.8% and 11.5% respectively.

Results of antimicrobial susceptibility testing

As shown in Table 5, the antimicrobial sensitivity test of Gram-negative bacteria isolates against 10 antibiotics related to 8 antimicrobial groups show that 80% of *Salmonella* sp. isolates were resistant to Cefotaxime and Colistin while 50%, 30% and 20% of isolates were resistant to Gentamicin, Tetracycline and Cefepime respectively, while 40% of *Salmonella* sp. isolates were resistant to Chloramphenicol, Enrofloxacin and Amoxicillin-clavulanate. While 60% of isolated *Salmonella*e showed resistant to Trimethoprim sulfamethoxazole and Ciprofloxacin.

As shown in Table 6, the antimicrobial sensitivity test of *E. coli* isolates showed that 91.6% and 83.3% were resistant to Cefotaxime and Colistin respectively, while 75% were resistant to Gentamicin and Tetracycline. While 50% of isolates were resistant to Ciprofloxacin, Enrofloxacin and Trimethoprim sulfamethoxazole. Also 58.3%, 25% and 8.3% were resistant to Chloramphenicol, Amoxicillin-clavulanate and Cefepime respectively.

As shown in Table 7, the antimicrobial sensitivity test of *P. aeruginosa* showed that 60% were resistant to Amoxicillin-clavulanate, Cefotaxime, Tetracycline Trimethoprim sulfamethoxazole, Chloramphenicol and Colistin, while *Proteus* sp. was 50% resistant to Gentamicin, Tetracycline, Amoxicillin-clavulanate, Cefotaxime and Colistin. While 30% of K. pneumonia was resistant to Cefotaxime, Gentamicin and Colistin. Also 20% of *Aeromonas hydrophila* was resistant to Cefotaxime and Colistin. 10% of *Y. enterocolitica* was resistant to Cefotaxime while 10% of *Citrobacter* sp. showed intermediate resistant to Cefotaxime.

Results of PCR for detection of antimicrobial resistance genes

The results revealed that bla_{TEM} and bla_{SHV} genes could be detected and amplification could be observed on the extracted DNA of 100 % of 12 isolates.

(*S.* Typhimurium, *S.* Enteritidis, *S.* Virchow, *S.* Kentucky, O114, O27, O158, O157, O119, *K. pneumoniae*, *P. aeruginosa* and *Proteus* sp.)

while bla_{CTX-M} gene could be detected and amplification could be observed on the extracted DNA of 41.6% of 12 isolates (*S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, O119 and *P. aeruginosa*). Genotypically, Figs. 1-3 showed the results of detection of resistance genes (bla_{TEM}, bla_{CTX-M} and bla_{SHV}) in Gram negative bacterial isolates.

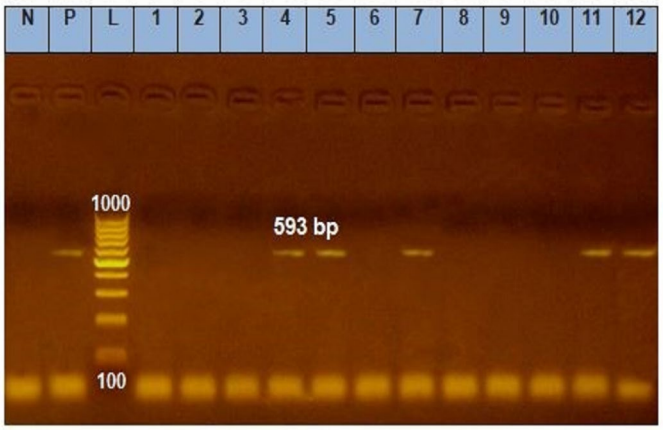

Fig.1. Detection of Beta-lactamase gene occurring among isolate*S.* Agarose gel electrophoresis of PCR: Amplification profile of bla_{CTX-M} gene at 593 bp for bacterial isolate*S.* Lane L: 100-1000 bp DNA ladder. N: Negative control, P: Positive control. Lane: 4,5,7,11 &12 (*S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, O119 and *P. aeruginosa*) were positive for bla_{CTX-M} gene. Lane: (*S.* Virchow, O114, O27, O158, O157, *K. pneumoniae*, *P. aeruginosa* and *Proteus* sp.) were negative.

Table 4. Prevalence of *E. coli* serogroups from positive pets' animal food (n= 23) and pets' fecal swabs (n= 26).

E coli serogroups	Pet food (n= 23)		Fecal swabs of pet (n= 26)		
	No.	%	No.	%	
O158	7	30.4	8	30.8	0.98
O157	5	21.7	7	26.9	0.674
O26	3	13	2	7.7	0.655 (*FET*)
O119	1	4.3	1	3.8	1.000 (*FET*)
O86	1	4.3	0	0	
O27	2	8.7	0	0	
O44	1	4.3	0	0	
O55	1	4.3	0	0	
O78	2	8.7	0	0	
O114	0	0	4	15.4	
O111	0	0	1	3.8	
O125	0	0	3	11.5	
p- value (*FET*)		0.045		0.022	

FET: Fishers Exact test.

Table 5. Antimicrobial sensitivity test of *Salmonella* isolate*S.*

Groups	Antimicrobial agent (micrograms)	Strains									
		S. Typhimurium	*S.* Virchow	*S.* Anatum	*S.* Kentucky	*S.* Kedougou	*S.*infantis	*S.* Nitra	*S.*ibargi	*S.* Enteritidis	*S.* Boecker
β-lactam/β-lactamase inhibitor combination	Amoxicillin-clavulanate (AMC 30)	S	R	S	S	I	R	R	R	S	S
Cephems	Cefepime (FEP 30)	S	S	R	S	R	S	S	S	S	I
	Cefotaxime (CTX 30)	R	R	I	R	R	R	R	R	R	S
Aminoglycosides	Gentamicin (CN 10)	S	R	I	R	I	R	S	S	R	R
Tetracyclines	Tetracycline (TE 30)	S	R	S	S	S	R	S	S	S	R
Quinolones and fluoroquinolones	Ciprofloxacin (CIP 5)	R	R	I	R	I	R	I	R	I	R
	Enrofloxacin (ENR 5)	R	R	S	R	S	S	S	S	S	R
Folate pathway inhibitors	Trimethoprim sulfamethoxazole (SXT 25)	S	R	R	R	R	R	S	S	S	R
Phenicols	Chloramphenicol (C 30)	S	S	S	R	R	R	S	S	S	R
Lipopeptides	Colistin (CT 10)	R	R	S	S	R	R	R	R	R	R

Table 6. Antimicrobial sensitivity test of *E. coli* serogroup*S.*

Group	Antimicrobial agent (micrograms)	Strains											
		O158	O157	O26	O119	O86	O27	O44	O55	O78	O114	O111	O125
β-lactam/β-lactamase inhibitor combinations	Amoxicillin-clavulanate	S	S	S	R	S	S	I	I	S	I	I	I
Cephems	Cefepime	S	S	S	S	S	S	R	R	S	R	S	S
	Cefotaxime	R	R	S	R	R	R	R	R	R	R	R	R
Aminoglycosides	Gentamicin	R	R	S	R	I	R	R	R	R	R	I	R
Tetracyclines	Tetracycline	R	S	R	R	R	R	R	R	S	R	R	S
Quinolones and fluoroquinolones	Ciprofloxacin	I	S	R	R	S	I	R	R	I	R	R	I
	Enrofloxacin	I	S	R	I	S	R	R	R	S	R	R	I
Folate pathway inhibitors	Trimethoprim sulfamethoxazole	S	I	R	S	S	R	R	R	S	R	R	I
Phenicols	Chloramphenicol	R	S	R	R	S	R	R	R	S	R	I	I
Lipopeptides	Colistin	R	S	S	R	R	R	R	R	R	R	R	R

Table 7. Antimicrobial sensitivity test of different bacterial isolate*S.*

Groups	Antimicrobial agent (micrograms)	Strains					
		Klebsiella pneumonae	*Proteus sp*	*Pseudomonas aeruginosa*	*Aeromonas hydrophila*	*Citrobacter sp*	*Yersinia enterocolitica*
β-lactam/β-lactamase inhibitor combinations	Amoxicillin-clavulanate	I	R	R	I	S	I
	Cefepime	S	S	S	S	S	S
Cephems	Cefotaxime	R	R	R	R	I	R
Aminoglycosides	Gentamicin	R	R	S	S	S	S
Tetracyclines	Tetracycline	S	R	R	S	S	S
Quinolones and fluoroquinolones	Ciprofloxacin	I	S	S	S	S	I
	Enrofloxacin	S	S	S	S	S	S
Folate pathway inhibitors	Trimethoprim sulfamethoxazole	S	I	R	S	S	S
Phenicols	Chloramphenicol	I	I	R	S	S	S
Lipopeptides	Colistin	R	R	R	R	S	S

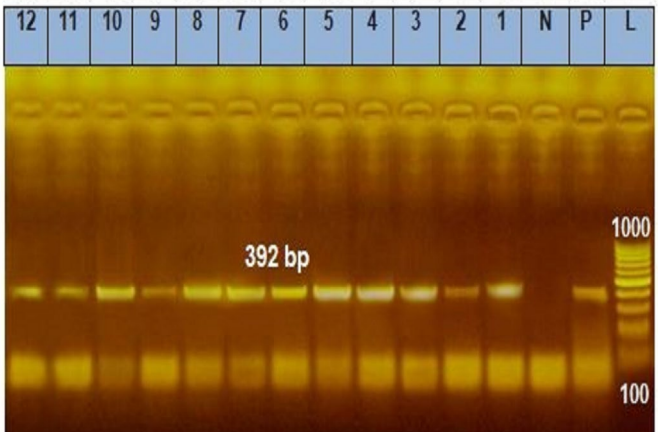

Fig 2. Agarose gel electrophoresis of PCR: Amplification profile of bla_{SHV} gene at 392 bp for bacterial isolate*S.* Lane L: 100-1000 bp DNA ladder. N: Negative control, P: Positive control. Lane: 1-12 (*S.* Typhimurium, *S.* Enteritidis, *S.* Virchow, *S.* Kentucky, O114, O27, O158, O157, O119, *K. pneumoniae*, *P. aeruginosa* and *Proteus* sp.) were positive for bla_{SHV} gene.

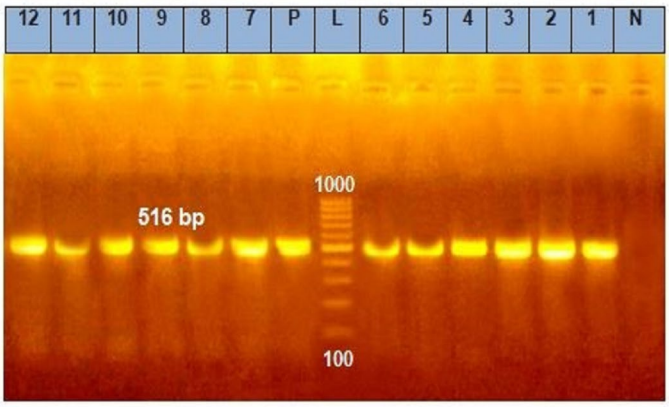

Fig.3. Agarose gel electrophoresis of PCR: Amplification profile of bla_{TEM} gene at 516 bp for bacterial isolate*S.* Lane L: 100-1000 bp DNA ladder. N: Negative control, P: Positive control. Lane: 1-12 (*S.* Typhimurium, *S.* Enteritidis, *S.* Virchow, *S.* Kentucky, O114, O27, O158, O157, O119, *K. pneumoniae*, *P. aeruginosa* and *Proteus* sp.) were positive for bla_{TEM} gene.

DISCUSSION

Infections caused by multidrug-resistant Gram-negative bacteria have been reported as one of the most strenuous growing problems worldwide.

In this study, the percentage of Gram-negative bacteria isolated from pet fecal swabs was 56%, while Hamame *et al.* (2022) found that Gram negative bacteria isolated from pet fecal swabs was 25% (30 out of 120) in cats and 50% (34 out of 68) in dogs' fecal samples.

E. coli was the most isolated bacteria in percentage of 12.4% from pet food and 25% from pet fecal swabs, this was almost agree to Mekky *et al.* (2021) in which they detected E coli in 45 cases out of examined 150 (30%) fecal samples of dogs and cats while it was lower than Marchetti *et al.* (2021) who reported that 95 out of 197 rectal swabs from dogs (48%), were biochemically identified as *E. coli* but lower than the study results of AbdAl-Rudha *et al.* (2020) who isolated 131 isolates from all fecal samples of 84 dogs, 50% were identified as *E. coli*, and lower than the study of Zarea *et al.* (2021) who revealed that the occurrence rate of *E. coli* was 67.7% (61/90) in rectal swabs of dogs (suffered from diarrhea with fever, nausea, chills, loss of appetite, and bloating) at different veterinary hospitals and clinics in Cairo.

In this study, the sero-grouping of *E. coli* revealed that O158 was the most predominant *E. coli* serotype isolated from pet food and pet fecal swabs in percentage of 30.4% and 30.8% respectively followed by O157 in percentage of 21.7% and 26.9% respectively. O26 was isolated serogroup from pet food and pet fecal swabs in percentage of 13% and 7.7% for each. O119 was

isolated serogroup from pet food and pet fecal swabs in percentage of 4.3% and 3.8% for each. O86, O27, O44, O55 and O78 were isolated serotypes from pet food in percentage of 4.3%, 8.7%, 4.3%, 4.3% and 8.7% respectively. O114, O111 and O125 were isolated serogroup from pet fecal swabs in percentage of 15.4%, 3.8% and 11.5% respectively. This almost agree with(Mekky *et al*. (2021) who Detected O157, O126, O114, O18, O26, O158, O111 and O18 in their study and Zarea *et al*. (2021) who carried out sero-grouping on ten *E. coli* isolates and concluded that they belonged to seven serogroups O18, O27, O55, O126, O148, O158, and O166 and other un-typable three strains.

In the present study, *Salmonella* sp. isolated from pet food was 0.6% while it was 5% from pet fecal swabs, it was revealed that the most predominant *Salmonella* serotype isolated from pet food and pet fecal swabs were *S.* Typhimurium (30.8% and 20%) respectively. while *S.* Virchow, *S.* Anatum, *S.* Kentucky, *S.* Kedougou and *S.* Infantis were isolated serotypes from pet food in percentage of 15.7%, 23.1%, 15.4%, 7.7%, 7.7% respectively. This was agreed with Viegas *et al*. (2020) who found that one dog (1/192 = 0.5%) fed commercial dry feed was positive for *Salmonella* spp. and some of the serovars detected were commonly associated with human salmonellosis, such as *S.* Typhimurium. This study almost agrees with the study of Usmael *et al*. (2022) who found 26 out of 415 (6.3%) dogs rectal swab samples were positive for *Salmonella* by using standard bacteriologic culture and biochemical tests.

In the current study, detection of *S.* Typhimurium almost agreed with the study of AbdAl-Rudha *et al*. (2020) who isolated 131 isolates from all fecal samples of 84 dogs,17% was identified as *S.* Typhimurium. Although, the study of Mekky *et al*. (2021) showed that 40 out 150 (26.6%) fecal samples of dogs and cats were positive for *Salmonella* (this more than the present study), but the identified serotypes were *S.* Typhimurium, *S. Entertidis*, *S.* Nitra, *S. Bocker*, *S. Entertidis* and *S.* Ibargi (as in this study). On the other hand, the prsent study was lower than Nemser *et al*. (2014) who determined that 15 out of 1056 raw pet food samples (1.4%) were positive for *Salmonella* and Yukawa *et al*. (2022) who detected *Salmonella enterica* subsp. enterica in seven of the 60 raw med-based diet samples (11.6%). Among them, five isolates were identified as *S.* Infantis (n = 3/42.9%), *S.* Typhimurium (n = 1/14.2%) and *S. Schwarzengrund* (n = 1/14.2%), while the serotypes of two isolates were unable to be identified (n=2/28.6). While the detection of *Salmonella* sp. from fecal samples was higher than the study of (Bataller *et al*., 2020) who clarified that 6 out of 325 dogs rectal sampled (1.85%) were positive for *Salmonella* sp. with 3 different serotypes, *S. Havana* (n=3) (50%), *S. Mikawasima* (n=2) (33.3%) and monophasic *S.* Typhimurium (n=1) (16.7%).

The current study disagreed with the study of Kazimierska *et al*. (2021) who examined thirty-six samples of dog dry food microbiologically, their study reported that none of the analyzed foods containing *Enterobacteriaceae*, including coliforms, *E. coli* and *Salmonella* sp.

K. pneumoniae, *Proteus* sp., *Enterobacter cloacae*, *P. aeruginosa*, *Aeromonas hydrophila*, *Citrobacter* sp., *P. fleurecense*, *Y. enterocolitica* were isolated from pet food in an order of 4.9%, 8.4%, 3.8%, 3.5%, 3.2%, 2.6%, 2.6% and 2.1% respectively. While *K. pneumoniae*, *Proteus* sp., *Enterobacter cloacae* were isolated in an order of 12%, 7% and 7% respectively, with no detection of *P. aeruginosa*, *Aeromonas hydrophila*, *Citrobacter* sp., *P. fluorescens*, *Y. enterocolitica* in pet fecal swabs samples (0% for each) this disagreed with the study of AbdAl-Rudha *et al*. (2020) who clarified that *Proteus* sp. and *Klebsiella* sp. not detected in all fecal samples of 84 dogs.

In the present study, the antimicrobial sensitivity profile of *Salmonella*e (as shown in Table 5) was agreed with Mekky *et al*. (2021) who found that *S.* Ibargi and *S.* Enteritidis were sensitive to chloramphenicol and enrofloxacin in addition, *S.* Boecker was resistant to gentamicin and Trimethoprim sulfamethoxazole. It agreed with Yukawa *et al*. (2022) who found that *S.* Infantis was resistant to tetracycline and trimethoprim, and the result of *S.* Typhimurium which was resistant to ciprofloxacin. Moreover, the obtained results agree with Usmael *et al*. (2022) who detected that 58.3% of *Salmonella* isolates showed resistance to at least one of the tested antimicrobial agents. While the present study disagreed with the results of Bataller *et al*. (2020) who found that All isolates were susceptible to all tested antimicrobials (Ampicillin, cefotaxime, ceftazidime, gentamicin, Nalidixic acid, ciprofloxacin, azithromycin, tetracycline, trimethoprim-sulfamethoxazole, clostin and chloramphenicol) and disagreed with the study of Mekky *et al* (2021) who clarified that *S.* Typhimurium, *S. Entertidis*, *S.* Nitra, and *S.* Ibargi were resistant to gentamycin and trimethoprim/sulphamethaxazole. In addition to Yukawa *et al*. (2022) who reported that all isolates were susceptible to cefotaxime and gentamycin. Moreover, this study disagrees the study of Usmael *et al*. (2022) who clarified that all isolates were fully susceptible to gentamicin.

In the current study, antimicrobial sensitivity profile of *E. coli* isolates showed that 91.6% were resistant to Cefotaxime (as shown in Table 6) this was agreed with Marchetti *et al*. (2021) who observed that the level of resistance to 3rd generation cephalosporins was high and agree with Zarea *et al*. (2021) who showed that 95.1% *E. coli* isolates were resistant cefotaxime. The present study showed that 75% were resistant to Tetracycline and 50% of isolates were resistant to Trimethoprim sulfamethoxazole, this is lower than Zarea *et al*. (2021) who clarified that All *E. coli* isolates were resistant to tetracycline, trimethoprim/sulphamethoxazole (100% for each). While it was higher than Rodríguez *et al*. (2020) who detected that the 38% of *E. coli* isolates were resistant to tetracycline.

bla_{TEM} and bla_{SHV} genes were detected in 100 % of 12 isolates (*S.* Typhimurium, *S.* Enteritidis, *S.* Virchow, *S.* Kentucky, O114, O27, O158, O157, O119, *K. pneumoniae*, *P. aeruginosa* and *Proteus* sp.) while bla_{CTX-M} gene was detected in 41.6% of 12 isolates (*S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, O119 and *P. aeruginosa*) this almost agree to Baede *et al*. (2017) who found that ESBL gene types in raw pet food were $bla_{CTX-M-1}$, $bla_{CTX-M-3}$, $bla_{CTX-M-15}$, $bla_{CTX-M-32}$, $bla_{CTX-M-2}$, $bla_{CTX-M-14}$, bla_{CMY-2} and bla_{SHV-12}. Bla_{CTX-M} was the dominant ESBL gene type, often accompanied by a bla_{TEM-1} variant.

CONCLUSION

In the current study, most of the bacteria isolated from either the pet food or the pets' stool harbored highly pathogenic and drug resistant bacteria. The pathogens' shedding in the pet's stool, contaminates the environment and may result in serious infections for both human and animals, via the hand to mouth route. Hygienic practices should be promoted to the public, especially after handling dog feces and raw meat. Pets' food should be properly cooked, and their dry food industry should undergo microbiological quality assurance and inspection before introducing to the market. These are important issues to be considered while a person is willing to own or deal with a pet animal.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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