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**Phenotypic and genotypic characteristics of antimicrobial resistant
Gram-negative bacteria isolated from pet animals and feedstuffs**

A thesis submitted by

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(Microbiology)**

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Abstract

Animal feed is highly susceptible to the introduction of bacteria throughout the entire production process. In this study, a total of 2948 samples collected from (2100 pet food, 100 pets' fecal swabs of diseased and apparently healthy dogs and cats, 227 feed additives, 367 fish meal, 29 wastes of poultry slaughter house meal, 47 calves milk replacer, 41 beet gain, 37 bone meal) were submitted to the serology unit, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt from 2017 to 2020. Only 1570 Out of 2948 examined pet animal and animal feedstuff samples (53.3%) were positive to the isolated Gram-negative bacteria, the incidence of isolated Gram negative bacteria among the collected sample types were identified biochemically to *E coli*, *Salmonella* sp., *Proteus* sp, *Klebsiella* sp.,

Yersinia enterocolitica, *Pseudomonas* sp, *Citrobacter* sp, *Enterobacter cloacae*, *Aeromonas hydrophila*. *E coli* isolates were the highest isolated Gram negative bacteria. The antimicrobial sensitivity test revealed that 80% of *Salmonellae* 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 *Salmonellae* were resistant to Chloramphenicol, Enrofloxacin, and Amoxicillin-clavulanate. Also 60% of *Salmonellae* showed resistance to Trimethoprim sulfamethoxazole and Ciprofloxacin. Detection of Extended-spectrum β -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 12 samples (41.6 %). It is desirable for laboratories to maintain bacterial strains in culture for extended periods of time for research purposes. Moreover, evaluation of the survival rate of these species of microorganisms after different preservation time (3, 6, 9 and 12 months) on soft agar, PBS with glycerol and cryopreservation in preservation temperature (4, -20 and -80°C) showed that the lowest survival rate after preservation on soft agar tubes preserved in 4°C, followed by preservation on phosphate buffer saline with glycerol preserved in -20°C, while the recovery rate after cryopreservation in -80°C were 100% for all isolates. The method described for preservation of Gram negative bacteria was a simple and economically useful for laboratories not equipped with the lyophilizer or ultra-low freezer.

Keywords: animal feedstuffs, Gram negative bacteria, *Salmonella* serovars, *E. coli* serogroups, Pets, Antimicrobial resistance, ESBL, *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}, preservation, cryopreservation.

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LIST OF ABBREVIATIONS

| | |
|-------|--|
| AMR | Antimicrobial resistance |
| CDC | Centers for Disease Control and Prevention |
| CFU | Colony Forming Unite |
| ISO | International Stander Organization |
| RASFF | Rapid Alert System for Food and Feed |
| WHO | World health organization |

Summary

Animal feed is highly susceptible to the introduction of bacteria throughout the entire production process. In this study a total of 2948 samples from (2100 pet food, 100 pets' fecal swabs of diseased and apparently healthy dogs and cats, 227 feed additives, 367 fish meal, 29 wastes of poultry slaughter house meal, 47 calves milk replacer, 41 beet gain, 37 bone meal) were submitted to the serology unit, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt from 2017 to 2020. Only 1570 Out of 2948 examined pet animal and animal feedstuff samples, (53.3%) were positive for the isolated Gram-negative bacteria, the incidence of isolated Gram negative bacteria among the collected sample types were identified biochemically as *E coli*, *Salmonella* sp., *Proteus* sp, *Klebsiella* sp., *Yersinia enterocolitica*, *Pseudomonas* sp, *Citrobacter* sp, *Enterobater cloacae*, *Aeromonas hydrophila*. *E coli* isolates were the highest isolated Gram negative bacteria. 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 with an incidence 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 *Salmonellae* 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 *Salmonellae* were resistant to Chloramphenicol, Enrofloxacin, and Amoxicillin-clavulanate. Also 60% of *Salmonellae* showed resistance to Trimethoprim sulfamethoxazole and Ciprofloxacin. Detection of Extended-spectrum β -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 12 samples (41.6 %).

It is desirable for laboratories to maintain bacterial strains in culture for extended periods of time for research purposes. Moreover, evaluation of the survival rate of these species of microorganisms after different preservation time (3, 6, 9 and 12 months) on soft agar, PBS with glycerol and cryopreservation in preservation temperature (4, -20 and -80°C) showed that the lowest survival rate after preservation on soft agar tubes preserved in 4°C, followed by preservation on phosphate buffer saline with glycerol preserved in -20°C, while the recovery rate after cryopreservation in -80°C were 100% for all isolates. The method described for preservation of Gram negative bacteria was a simple and economically useful for laboratories not equipped with the lyophilizer or ultra-low freezer.