

## The Prevalence of Aerobic Bacteria Isolated from Horses Fecal Samples

Aseel, M.Hamzah; Jenan, M., Khalef; Ibrahim, A. Al-Zubaidy  
Zoonotic disease unit / veterinary college / Baghdad University  
Email: aseelm30@yahoo.com Ibrahim\_hussein2000@yahoo.com

### Abstract

Dietary components and changes cause shifts in the gastrointestinal microbial ecology that can play a role in animal health and a wide range of diseases. However, most information about the microbial populations in the gut of horses has not been quantitative. The objective of this study was to characterize the fecal bacterial and its prevalence in healthy horses and diarrheal one in a period from September 2010 to July 2013. Out of 100 Fecal samples of horses (from farms in Al-furusyia club) in Baghdad were examined for microbial differentiation founded eighty percent of the fecal samples isolated from healthy horses. The most common pathogen found were *Streptococcus* spp. (33.7%), *Escherichia coli* (20.9%), , and *Staphylococcus aureus* (9.2%). Relatively low frequency of detection was found for *serratia marcescens* spp. (0.6%), *Acintobacter* spp.(1.2%), (1.8%) for *Enterococcus* sp., *Micrococcus* sp., *Proteus vulgaris*, *Klebsiella pneumoniae* and *Citrobacter diversus* , *Pseudomonas* spp (2.5%), *Salmonella* spp. (3.1%), and *Listeria monocytogenes* (0.0%) .

**Key words:** Fecal samples, Aerobic bacteria, Isolation, Horses.

### Introduction

The animal gastrointestinal (G.I.) tract maintains a rich microbial community in which directly affects energy metabolism, digestive function, mucosal immune system development, and disease pathogenesis of its eukaryotic host [1-4].

The homeostatic balance in the equine intestinal microbiome is very sensitive to factors like gastrointestinal disease and dietary change, which may lead to catastrophic consequences, even culminating in death [5,6].

Alterations in hindgut bacterial communities have also been associated with several equine diseases [7-14] else diseases affecting the gastro-intestinal system are the main cause of mortality in this species [5].

Numerous factors including the evolutionary history of the host, age, and diet influence the diversity of gut microbes [2] in addition to Starch and oligofructose overload-induced models have revealed strong associations between onset of laminitis and proliferation of *Streptococcus* and *Lactobacillus* bacteria, with a concurrent decrease in intraluminal pH [9-12,15-18].

Patterns of microbial diversity in the G.I. tract have important implications for human and environmental health. These communities become a source of pathogens when released into aquatic environments as fecal pollution. Community members that exhibit host-specific distributions represent a valuable resource as potential markers for fecal pollution from specific sources. Accordingly, characterization of the equine intestinal microbial is critical, since a good understanding of the 'normal' intestinal microbial is needed for interpretation of 'abnormal'. Most investigations of the equine microbial have typically involved bacterial culture of feces or intestinal contents.

The objectives of this study were to characterize the fecal microbial of healthy horses.

### Material and methods

**1-Animals:** - One hundred horses of different breeds, ages, and sex were included in the study.

**2-Sample collection:** - For the microbiological survey, 100 fecal samples were collected from Horses at the Al-Furusyia club. The animals (with ages ranging from young to mature) were predominating; fecal samples were collected from the rectum with sterile gloves put in sterile cleaning cup with screw and arrival at the laboratory of zoonotic disease unit in Veterinary Medicine College of Baghdad University.

**3-Sample inoculation:** -approximately 10g or mL of feces was obtained rectally from horses the fecal materials were collected into sterile fecal cups .The faecal samples (liquid, semisolid, and solid) were then processed upon arrival or within 24 h of collection whenever feasible.

Fecal samples inoculated on enrichment media using blood agar after incubated for 24 hours at 37°C then using Gram stain to characterized Gram Negative and Positive bacteria after that inoculated on different selective media using MacConkey agar, Listeria agar (BALCAM) with supplement , manitol salt agar, Edward agar, Staph 110 agar, pseudomonas agar then Conventional biochemical methods according to standard microbiological techniques [19] was done for bacteria characterized, in addition tetrathionate broth enrichment with subsequent subculture on *Salmonella-Shigella* agar, Xylose Lysine Deoxycholate (XLD) and brilliant green agar was performed to attempt isolation of *Salmonella* sp.

**4. Determination of Resistance to Antimicrobial Agents:-** The resistance of bacterial isolates to antimicrobial agents was determined using the disc diffusion method. For the study, the following antimicrobial agents and concentrations were used: gentamicin (CN,10µg), Tetracycline(TE, 30µg), chloramphenicol (C, 30µg),sulphamethoxazole/trimethoprim (SXT, 23.25µg/1.75µg),streptomycin (S, 10µg) and ampicillin (AMP, 10µg). The breakpoints of the National Committee for Clinical Laboratory Standards, NCCLS [20], were used to determine the susceptibility or resistance of isolates to the antimicrobial agents

## Results

**1. Frequency of Detection of aerobic bacterial in Fecal Samples:-** A total of 100 fecal samples were collected from horses during the September 2010 to July 2013; however, only 9 had ongoing diarrhea during the study period. The result Classify most bacteria into two main groups: Gram-positive and Gram negative. Bacteria from all two groups were found in normal equine flora ,The frequency was comparatively high for *Streptococcus* spp. (33.7%), *Escherichia coli* (20.9%), , and *Staphylococcus aureus* (9.2%) as shown in Table 1. Relatively low frequency of detection was found for *Serratia marcescens* spp. (0.6%), *Acintobacter spp.*(1.2%), (1.8%) for *Enterococcus sp.*, *Micrococcus sp.*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Citrobacter diversus* , *Pseudomonas spp* (2.5%), *Salmonella* spp. (3.1%), and *Listeria monocytogenes* (0.0%) as shown in Table 1.

**Table (1) Bacterial species isolated from the fecal samples of equines**

Type of bacteria	Total number of isolate	% of isolates
<b>Gram-positive</b>	<b>101</b>	<b>61.9%</b>
<i>Bacillus spp.</i>	12	7.4%
<i>Nocardia spp.</i>	9	5.5%
<i>Rhodococcus equi</i>	11	6.7%
<i>Staphylococcus aureus</i>	15	9.2%
<i>Streptococcus spp.</i>	55	33.7%
<i>Enterococcus sp.</i>	3	1.8%
<i>Listeria monocytogenes</i>	0	0%
<i>Micrococcus</i>	3	1.8%
<b>Gram-negative</b>	<b>55</b>	<b>37.4%</b>
<i>Escherichia coli</i>	34	20.9%
<i>Salmonella spp.</i>	5	3.1%
<i>Proteus vulgaris</i>	3	1.8%
<i>Pseudomonas spp</i>	4	2.5%
<i>Klebsiella pneumoniae</i>	3	1.8%
<i>Acintobacter sp.</i>	2	1.2%
<i>Serratia marcescens</i>	1	0.6%
<i>Citrobacter diversus</i>	3	1.8%
<b>total</b>	<b>163</b>	<b>100%</b>

**2.Frequency of bacterial isolates in Diarrheic and Non diarrheic horses:-** The frequency of isolation of *Salmonella* spp. in diarrheic horses (33.3%), that is, 3 of 9 horses, higher than found in non-diarrheic horses (2.0%), that is, 2 of 100 horses as shown in Table (2).

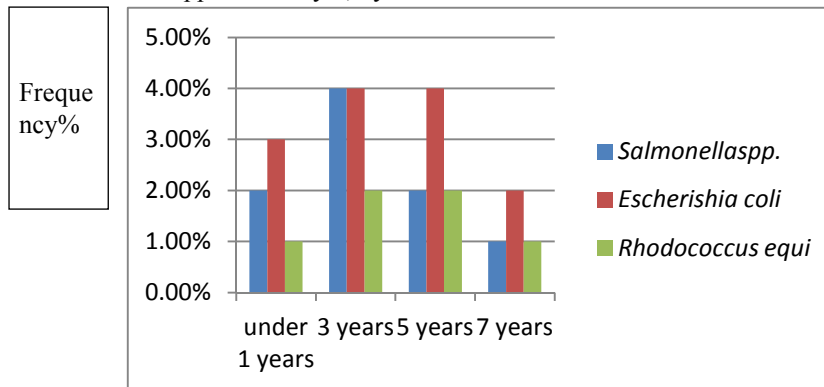
*E. coli* was isolated at a higher rate (100.0%) in diarrheic horses compared with non-diarrheic horses (20.3%) else *Rhodococcus equi* (22.2%) in diarrheic horses and (9.0%) in non-diarrheic horses However, the frequency of detection of *E. coli* , *Salmonella* spp and *Rhodococcus equi* was found to be higher in diarrheic horses compared with non-diarrheic horses.

**Table (2) Frequency of bacterial isolates in Diarrheic and Non diarrheic horses**

Type of bacteria	Diarrheic horses		Non-diarrheic horses	
	Number of horses test	Number(%) positive	Number of horses test	Number(%) positive
<i>Escherichia coli</i>	9	9 (100%)	100	25 (20.3%)
<i>Salmonella spp.</i>	9	3 (33.3%)	100	2 (2.0%)
<i>Rhodococcus equi</i>	9	2 (22.2%)	100	9 (9.0%)

**3. Frequency of Detection of Diarrheic bacterial isolates by Age of horses:-** The age of the horses population was normally distributed with a range of 4 month to 7 years (Figure 1).

Five (55.6%) of the 9 horses which had diarrhea at the time of sample collection were approximately 4-12 month old while the remainder were approximately 3,5 years old.



**Figure 1: Frequency of detection of diarrheic pathogens in horses by age.**

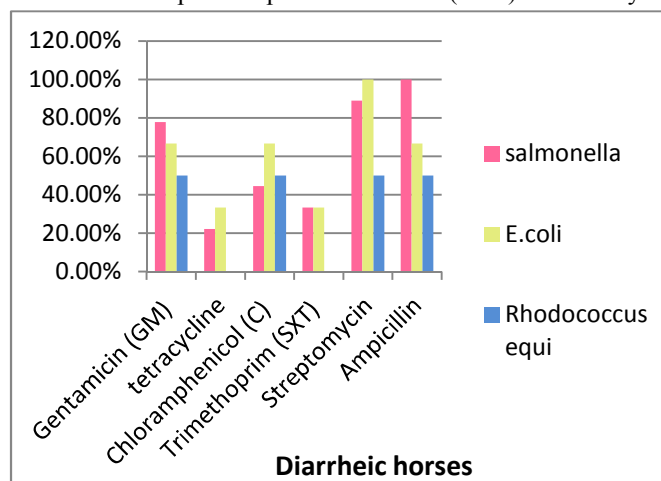
**4. Frequency of Detection of bacterial isolated by Sex of horses.**

For the 100 horses in the sample population whose sex was known, 57 (57%) were female while 43 (43%) were male. The majority (57%) that is races farms had a higher number of female horses. Bacterial isolated Higher in Female than in male.

**4. Prevalence of Resistance of E.coli, Salmonella spp. Rhodococcus equi and isolates to Antimicrobial Agents.**

The numbers of resistant cultures to each antibiotic, expressed as a percent of the total cultures tested for each antibiotic are graphed in Figure 2 of diarrheic horses. For the 6 antimicrobial agents tested, Ampicillin was the most effective antibiotic used in diarrheic horses. For *E.coli* from diarrheic horses non isolates have antibiotic sensitivity inhibition zones for Ampicillin indicated that the sampled organisms were resistant to this antibiotic. Streptomycin was the second most effective antibiotic with only 8 of the *E.coli* isolates resistant. Gentamicin (GM) was third 7 isolates resistant, Chloramphenicol (C) with 4, Trimethoprim/Sulphamethoxazole (SXT) also with 3 isolates, and lastly Tetracycline with 2 isolates resistant. While *Salmonella* spp for diarrheic sample the resistant strain for antibiotic is 3 resistant for streptomycin, 2 resistant for Gentamycin, Ampicillin and chloramphenicol for all and 1 resistant for Trimethoprim/Sulphamethoxazole (SXT) and Tetracycline.

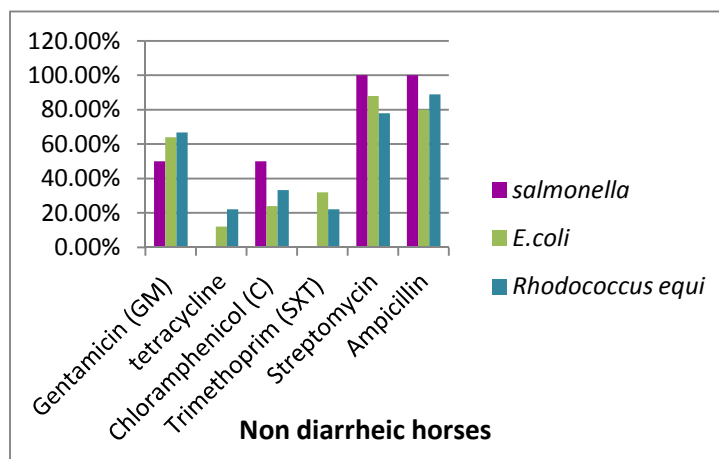
*Rhodococcus equi* was one resistant for Ampicillin, Streptomycin, chloramphenicol, chloramphenicol, Gentamycin while sensitive for Trimethoprim/sulphamethoxazole (SXT) and tetracycline.



**Figure(2) Percent of resistant antibiotic sensitivity inhibition zones. Percent's reflect the number of resistant isolates out of the total bacteria isolates resistant to the given antibiotic in diarrheic horses.**

The percent of resistant to antibiotic in healthy horses are graphed in Figure 3, for *E.coli* was 22,20,16,8,6,3 resist isolates for Streptomycin, Ampicillin, Gentamycin, Trimethoprim/Sulphamethoxazole (SXT), chloramphenicol and Tetracycline consequently, while number of *Salmonella* spp resistant isolates was 3 for

Streptomycin, Ampicillin, 2 resistant isolates for chloramphenicol, Gentamycin and nonresistant isolates for Trimethoprim/Sulphamethoxazole (SXT) and Tetracycline, while in *Rhodococcus equi* resistant isolates was 8,7,6,3 for Ampicillin, Streptomycin, Gentamycin, chloramphenicol and two resistant isolates for Tetracycline respectively.



**Figure(3) Percent of resistant antibiotic sensitivity inhibition zones. Percent's reflect the number of resistant isolates out of the total bacteria isolates resistant to the given antibiotic in healthy horses.**

The frequency of resistance was higher for isolates recovered from diarrheic than from non-diarrheic horses (Table 3).

**Table 2: Frequency of resistance bacterial isolates to antimicrobial agents.**

Animal status	type of bacterial isolates	Number of isolates	Number % of Isolate resistant to antimicrobial agents					
			CN	TE	C	SXT	S	A
Diarrheic horses	<i>E.coli</i>	9	77.8%	22.2%	44.4%	33.3%	88.9%	100%
	<i>Salmonella spp</i>	3	66.7%	33.3%	66.7%	33.3%	100%	66.7%
	<i>Rhodococcus equi</i>	2	50%	0%	50%	0%	50%	50%
Non-diarrheic horses	<i>E.coli</i>	25	64%	12%	24%	32%	88%	80%
	<i>Salmonella spp</i>	2	50%	0%	50%	0%	100%	100%
	<i>Rhodococcus equi</i>	9	66.7%	22.2%	33.3%	22.2%	77.8%	88.9%

### Discussion

These results suggest that normal horse fecal flora includes a wide variety of organisms which demonstrated as a critical factor for normal GI function [21,22,23] on the other hand the bacterial populations differ related to horse's diet that composed of high fiber and reduced fat, protein, and digestible carbohydrates [24] and diet fermentation [7], else the fecal microbial of healthy horses showed that the equine microbial is more diverse than the human microbial, but less diverse than the gastrointestinal microbial of cattle [24].

Our result indicate the higher percentage for *Streptococcus spp.* Isolation (33.7%) that agreement with many studies [11, 25–27] this genus not cause laminitis in horses [17].

This study determined the rate of prevalence of bacterial agents associated with diarrhea in horse in Baghdad. The bacteriologic isolates found in horses in the present study include *E. coli*, *Salmonella* and *Rhodococcus equi* and these organism were previously reported by [28,29] in horses. During the present study the rate of prevalence of *E. coli* was highest than other pathogens (20.9%) which agree with other several studies, *E. coli* [30-36].

The bacterial isolation in diarrhea depends mainly on the age of animal and the surrounding environment as well as weather, management which act as predisposing factor on animal for inducing diarrhea, But in the study interested mainly on the bacterial isolation in a horse fecal sample admitted to veterinary diagnostic lab not concerned with animal age and other factors. So the presence of *E. coli* in large percent does not indicate

occurrence of diarrhea because it most common in the feces of foal [31] also adult horse shedding salmonella in the feces for long time and become source of infection [37]. The our finding that these enteropathogens were detected in non-diarrhoeic horses which were predominantly (25 of 100 horses) available for the study is in agreement with published reports [28, 7, 23].

Based on the antibiograms of the *E. coli* isolates in the current study, it is evident that they were mostly sensitive to chloramphenicol and Tetracycline but least susceptible to ampicillin and streptomycin. The detected resistance to both ampicillin and streptomycin could be explained, the fact that these antimicrobial agents were used at a high frequency on the study farms. Another finding in the current study with clinical relevance was the detection of higher frequency of resistance to tetracycline amongst isolates of *E. coli* recovered from diarrheic compared with non-diarrheic horses. This is an indication of a possible development of resistance due to misuse or overuse of the antibiotic in the treatment of horses locally.

The frequency of isolation (33.3%) of *Salmonella* spp. from diarrheic foals in the current study is higher than the rates of 12% (28/233) [36] and 13% (60/465) [38] reported for diarrheic foals elsewhere but lower than the 35.1% (34/97) reported by Walker et al. [39]. The fact that the rate of isolation of *Salmonella* spp. was significantly higher than found in non-diarrheic foals suggests etiological significance as earlier documented by others [36,40]. The finding that all isolates of *Salmonella* spp. recovered in this study were sensitive to both sulphathoxazole/trimethoprim (SXT) and Tetracycline is an indication that the two antimicrobial agents may be important in the chemotherapy of foal diarrhea caused by *Salmonella* spp.

Based on the questionnaire survey of the farmers during the study, the commonly used antimicrobial agents used to control foal diarrhea are streptomycin and ampicillin.

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