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THE ROLE OF GRAM NEGATIVE BACTERIA IN GYNAECOLOGICAL DISEASES IN ARABIAN MARES

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SUMMARY

A total of 171 samples were collected from Arabian mares suffering from gynaecological disorders included abortion (19) cases, endometritis (21) cases, pyometra (6) cases and repeat breader (125) cases, all the samples were examined bacteriologically. The incidence of Gram negative bacteria was 74.85%. Relatively high rate of abortion was seen among mares during winter and autumn. The most predominant bacterial species isolated from the examined samples were *E. coli* (60.82%), *C.freundii* (4.68%), *P.mirabilis* (2.34%), *P. aeruginosa* (2.34%), *E. intermedius* (1.17%), *K. pneumoniae* (1.17%), *Shigella species* (1.17%), *Providencia alcalifaciens* (0.58%) and *Serratia liquefaciens* (0.58%).

The antibiogram study on some selected isolates obtained from Arabian mares with gynaecological disorders showed that ciprofloxacin, danofloxacin and gentamicin were the most effective antibiotics against the isolated microorganisms.

INTRODUCTION

Recent development in equine medicine and reproduction has meant that an extra care needs to be assimilated towards equine.

Arabian horses were and still be extraordinary animals with global reputation for purity, beauty and outstanding value.

Breeding of Arabian mares usually faces troubles due to reproductive disorders particularly those caused by bacterial agents.

Gram negative bacteria mainly *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus mirabilis* (*P.mirabilis*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Shigella* species constitute the major bacterial causes of reproductive disorders in Arabian mares in Egypt including infertility, abortion and inflama tion of the different sites of the reproductive tract (El Baroudy, 1987 and Hatem et al., 1989a)

The present investigation was targeted to throw more light on the role of Gram negative bacteria in the reproductive disorders of Arabian mares in the largest (and probably one of the largest Arabian horse studs in the world (El- Zaharaa) and some other Arabian mares raised in small private studs in Cairo, Giza and El-Sharquia governorates. Determination of the causative agent/s of each gynaecological affection, abortion, identification of them, and antibiogram determination of important isolates were among our aim in the present study.

MATERIAL AND METHODS

1-Samples:

A total of 171 samples were collected from 167 Arabian mares as well as 4 swabs from the stomach contents of aborted feti as shown in Table (1), most of them were suffering from reproductive disorders as abortion, endometritis, pyometra and repeat breeder (with or without silent heat). These mares were raised on El-Zaharaa Stud, which were either originally kept and bred on such stud or belonged to private studs located in different governorates (Cairo, Giza and El - Sharquia) and brought about to the Veterinary Clinic of El-Zaharaa for matting and gynaecological examination.

The bacteriological examination of samples was carried out according to Krieg and Holt (1984); Quinn et al. (2002) and Winn et al.(2006).

2- Bacteriological examination:

Each sample was inoculated into a tube of peptone water, then it was incubated at 37°C for 24 hours. For selective isolation of salmonella, the incubated samples were inoculated into tubes of selenit F broth (Biomerieux) and incubated at 37°C for 14 - 18 hours.

- Plating on solid media :

The inoculated tubes were streaked onto MacConkey agar (Oxoid), nutrient agar (Oxoid) and 7 - 10% sheep blood agar plates. While for salmonella, the inoculated and incubated selenite F broth were streaked onto Salmonella- Shigella agar (S.S. agar) (Difco) and Brilliant green agar (Britania) plates. All the inoculated plates were incubated at 37°C for 24-48 hours, then were examined for colonial growth and the cultural characteristics which including the patterns of growth, colonial morphology, hemolysis on blood agar and pigment production on nutrient agar, as well as the characteristic growth on the selective differential media.

. Microscopical examination:

smears from parts of suspected colonies on the plates were prepared microscopically, fixed and stained with Gram's stain. The corresponding colonies of the detected Gram negative organisms were picked and subcultured onto nutrient agar slant (as pure culture), incubated at 37°C for 24 hours and kept for further biochemical identification .

Table (1): Total number and types of the examined samples collected from different affections of Arabian mares:

Affections	Type of Samples	Number of Samples
Abortion	Cervical swabs	15
	Stomach contents of aborted feti	4
Endometritis	Cervical swabs	21
Pyometra	Cervical swabs	6
Repeat breeder	Cervical swabs	125
Total		**171**

3- In Vitro antibiogram testing for some Gramnegative isolates:

An antibiogram study was carried out according to Forbes et al. (1998) and was conducted on 35 isolates of Gram negative bacteria obtained from cervical swabs of 34 diseased Arabian mares and one isolate from one aborted fetus. These isolates were selected randomly.

The disc diffusion antibiotic suscptibility test was carried out on the isolates of Gram negative bacteria for their susceptility to 15 antibacterial discs namely: amoxycillin (AML), ampicillin (AMP), amoxycillin/ clavulanic acid (AMC), chloramphenicol (C), erythromycin (E), ciprofloxacin (CIP), cloxacillin (OB), danofloxacin (DNF), enrofloxacin (ENR), flumequine (UB), gentamicin (CN), nalidixic acid (NA), penicillin (P), streptomycin (S) and tetracycline (TE).

The antibiotic sensitivity test was carried as follows:

A colony of the organism was transferred and emulsified into a tube of sterile Muller Hinton broth (Britania), mixed well and incubated at 37°C for 24 hours.

The turbidity of the inoculated broth was matched to be approximately equivalent McFarland tube number 0.5, the adjusted tubes were

streaked evently onto Muller Hinton agar plates (Britania) using a sterile cotton swabs. The inoculated plates were left for 5 - 15 minutes to dry.

The selected antibiotic discs (Oxoid) were plated on the surface of the inoculated plates and gently pressed onto the agar surface plates. The inoculated plates were incubated for 18 - 24 hours at 37°C. the diameters of complete inhibition zones were measured and the interpretation of the results was read according to NCCL (2002) and Winn et al. (2006).

RESULTS

1-Results of bacteriological examination and identification.

The results of bacteriological investigation carried out on 171 cases from the examined Arabian mares showing abortion, endometritis, pyometra and repeat breeder cases are shown in Tables (2 & 3) in which 128 Gram negative isolates were obtained in an incidence of 74.85%. *E.coli* constituted the most predominant organism from the total bacteria recovered from different diseased cases and aborted feti, it was recovered from 104 (60.82%) out of 171 cases.

C. freundii, 8 isolates (4.68%) were recovered from repeat breeder mares, as well as endometritis and pyometra cases.

P. mirabilis, 4 isolates (2.34%) were recovered from diseased Arabian mares.

Four isolates (2.34%) were identified as *P. aeruginosa*, of which three isolates were recovered from aborted mares and one from a mare showing endometritis.,

The two Enterobacter isolates (1.17%) were isolated from repeat breeder mares only.

Two isolates of *K. pneumoniae* (1.17%) were recovered from mares suffering from endometritis.

The two Shigella isolates (1.17%), one isolate was recovered from a case of endometritis and the other one from a repeat breeder mare.

The Providencia isolate (0.58%) was isolated from a cervical swab of a repeat breeder mare with apparently healthy genital tract.

The isolated Serratia liquefaciens (0.58%) was recovered from a repeat breeder mare.

According to the examined cases, the Gram negative bacteria constituted the highest incidence (116.67%) from samples of mares with pyometra, followed by samples of abortion (100%), Moreover, repeat breeder cases had an incidence of recovery of (69.60%), while cases of endometritis (71.43%) with respect to the total examined cases (Table 2) .

Gram positive bacteria were also recovered from cases of abortion, endometritis and repeat breeder mares, Gram positive cocci with a recovery rate of (3.51%) were represented by streptococci, staphylococci and micrococci. Whereas the recovery rate of Gram positive bacilli was (4.09%) as shown in Table (3) .

2-The incidence of abortion in Arabian mares along the four years of investigation during the different seasons to study the effect of seasonal variation on the rate of abortion.

2. 1. Table (4) and Figure (1) showed the case numbers of aborted Arabian mares belonging to El-Zaharaa Stud:

It is observed that the highest percentage of abor-

tion was detected during the winter and autumn seasons with a rate of (2.98%) and (2.71%) respectively. The rate declined during spring to (0.54%) while it reached minimal rate (0.27%) during summer season.

2. 2. Table (5) and Fig. (2) show the number of cases of aborted Arabian mares belonging to private studs.

It was noticed that the highest percentage of abortion was detected during the winter with the rate of (3.06%) and declined to (2.04%) and (1.02%) in autumn and spring. No abortion cases were recorded in summer.

It is of importance to mention that, the total number of pregnant mares belonging to El- Zahraa Stud during the period (2001- 2004) was 369 of which the aborted mares were 24 cases, whereas, the aborted cases of mares collected from private studs during this period were 6 cases out of 98 pregnant mares. Not all the aborted cases could be examined bacteriologically because not all the samples were available for examination.

3- In vitro antibiogram test for some Gram negative isolates:

The obtained results are given in Tables (6 &7), the 18 *E. coli.* isolates (100%) were sensitive to ciprofloxacin (CIP), danofloxacin (DNF), enrofloxacin (ENR) and gentamicin (CN), while 16 out of them (88.89%) were sensitive to chloramphenicol (C), flumequine (UB), and tetracycline (TE). 83.33% of the isolates were sensitive to amoxycillin/ clavulanic acid (AMC), erythromycin (E) and nalidixic acid (NA). *E. coli.* isolates were also susceptible to streptomycin (S), ampicillin (AMP) and amoxycillin (AML) showing susceptibility rates of 77.78%, 38.89% and 16.67%, respectively. On the other hand all of the isolates were fully resistant to cloxacillin (OB) and penicillin (P).

All the 6 *C. freundii* isolates (100%) were sensitive to C and CIP, while 5 (83.3%) were sensitive to DNF, ENR, UB, CN, NA, S and TE. 66.6% of the isolates were sensitive to AMC and AMP. Only 50% of the tested isolates were sensitive to AML. On the other hand, all of the isolates were fully resistant to OB and P.

In case of *P. mirabilis*, they were 100% sensitive to AMC, ENR, UB and CN, 66.66% of them were sensitive to CIP, DNF, E and NA, 33.33% were sensitive to AMP, C, S and TE. Whereas, *P. mirabilis* isolates were fully resistant to AML, OB and P.

The *P. aeruginosa* isolates were 100% sensitive to CIP, DNF and ENR. The isolates were also susceptible to S at rate of 66.66%, the susceptibility of them to AML, C, E, UB, CN, NA and TE was 33.33% for each. On the other hand, the isolates were fully resistant to AMC, AMP, OB and P.

In case of the two *K. pneumoniae* isolates, all of them (100%) were susceptible to 12 out of the 15 examined antibiotics as shown in Table (6) and 50% of them were sensitive to E, while all isolates were fully resistant to OB and P.

The two *Shigella spp.* isolates were sensitive 100% to 13 out of the 15 examined antibacterial agents as shown in Table (6). In contrary they were fully resistant to OB and P.

The *Providencia alcalifaciens* isolate was sensitive to most antibiotic discs applied except AML, OB and P as shown in Tables (6 & 7) .

Table (2): Numbers and types of bacteria recovered from different cases.

Mare's affections Numbers of cases (N=171)	Abortion (N=19)		Endometritis (N = 21)		Pyometra (N=6)		Repeat breeders (N=125)	
Recovered bacteria	No.	%	No.	%	No.	%	No.	%
1-Gram negative oxidase negative bacteria								
Escherichia coli	16	84.21	8	38.09	5	83.33	75	60
Citrobacter freundii	-	0	2	9.52	1	16.67	5	4
Proteus mirabilis	-	0	1	4.76	1	16.67	2	1.60
Enterobacter intermedius	-	0	-	0	-	0	2	1.60
Klebsiella pneumoniae	-	0	2	9.52	-	0	-	0
Shigella species	-	0	1	4.76	-	0	1	0.80
Providencia alcalifaciens	-	0	-	0	-	0	1	0.80
Serratia liquefaciens	-	0	-	0	-	0	1	0.80
Total oxidase negative	16	84.21	14	66.67	7	116.67	87	69.6
2- Gram negative oxidase positive bacteria								
Pseudomonas aeruginosa	3	15.79	1	4.76	-	0	-	0
Total Gram negative	19	100	15	71.43	7	116.67	87	69.60
3- Gram Positive bacteria								
Gram positive cocci	2	10.53	3	14.29	-	0	1	0.80
Gram positive bacilli	-	0	-	0	-	0	7	5.60

Table (3): Incidence of bacteria recovered from different affections in Arabian mares (171).

Isolated bacteria	Number	Percentage (%) (recovery rate)
Escherichia coli	104	60.82 %
Citrobacter freundii	8	4.68 %
Proteus mirabilis	4	2.34 %
Pseudomonas aeruginosa	4	2.34 %
Enterobacter intermedius	2	1.17 %
Klebsiella pneumoniae	2	1.17 %
Shigella spp.	2	1.17 %
Providencia alcalifaciens	1	0.58 %
Serratia liquefaciens	1	0.58 %
Total Gram negative bacteria	128	74.85 %
Gram positive cocci	6	3.51 %
Gram positive bacilli	7	4.09 %
Total Gram positive bacteria	13	7.6 %

Table (4): Prevalence of equine abortions (2001-2004) at El- Zahraa Stud [the rates were calculated according to the total number of pregnant mares (369)].

Season	Year / Month	2001	2002	2003	2004	Total/season No. (%)
Winter	December	2 (0.54%)	-	-	-	11 (2.98%)
	January	-	-	4 (1.08%)	1 (0.27%)	
	February	2(0.54%)	-	1(0.27%)	1(0.27%)	
Spring	March	-	-	-	-	2(0.54%)
	April	-	-	2(0.54%)	-	
	May	-	-	-	-	
Summer	June	-	-	-	-	1 (0.27%)
	July	-	1(0.27%)	-	-	
	August	-	-	-	-	
Autumn	September	-	-	3 (0.81%)	-	10 (2.71%)
	October	4 (1.08%)	-	-	1 (0.27%)	
	November	-	-	2 (0.54%)	-	

Table (5): Prevalence of equine abortions (2001 – 2004) at some private studs (the rates were calculated according to the total number of pregnant mares (98) of some private studs).

Season	Year / Month	2001	2002	2003	2004	Total/season
Winter	December	-	1 (1.02%)	-	1 (1.02%)	3 (3.06%)
	January	-	-	1 (1.02%)	-	
	February	-	-	-	-	
Spring	March	-	-	1 (1.02%)	-	1 (1.02%)
	April	-	-	-	-	
	May	-	-	-	-	
Summer	June	-	-	-	-	-
	July	-	-	-	-	
	August	-	-	-	-	
Autumn	September	-	-	-	-	2 (2.04%)
	October	-	-	-	-	
	November	2(2.04%)	-	-	-	

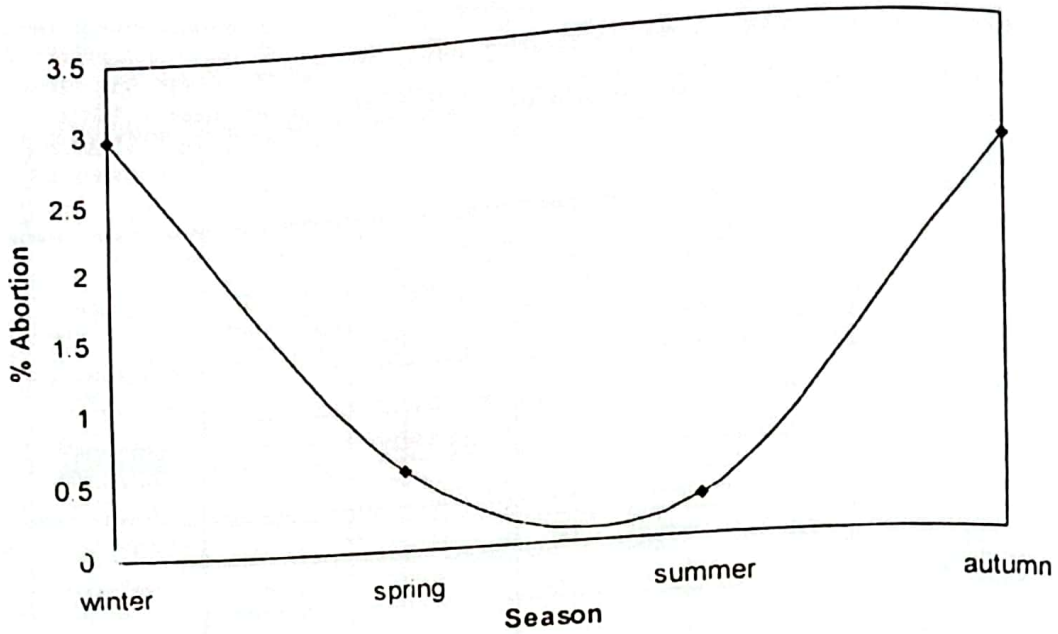

fig.1: A curve showing the prevalence of equine abortions in different seasons at Al-Zahraa Stud

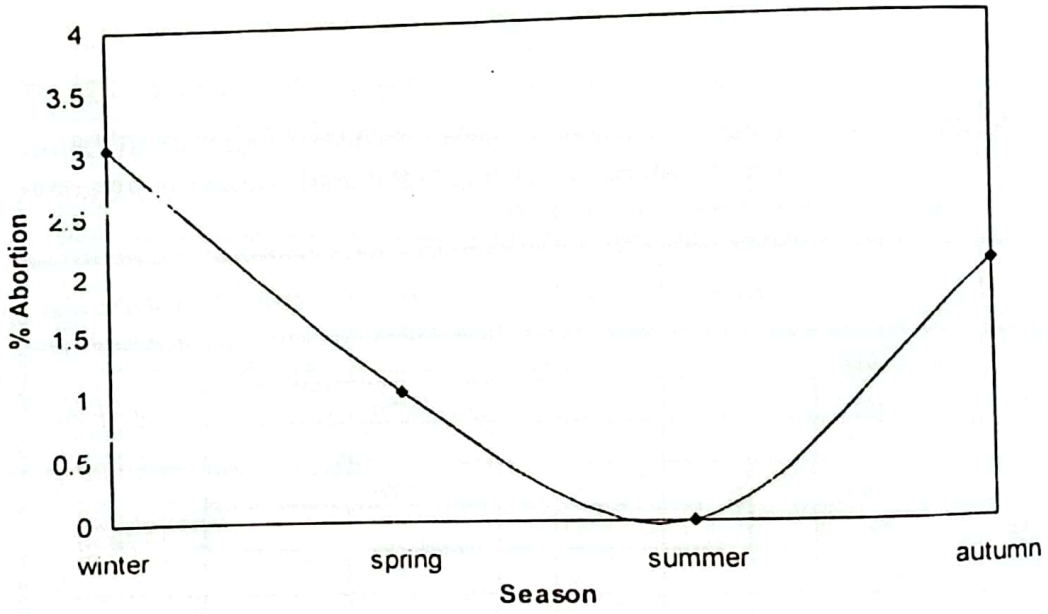

fig.2: A curve showing the prevalence of equine abortions in different seasons at some private studs

Table (6) : Number and percentage of sensitive bacteria recovered from Arabian mares and aborted feti :

Antibacterial Agents / Organisms	No. tested	AML		AMC		AMP		C		CIP		OB		DNF		ENR		E		UB		CN		NA		P		S		TE	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Escherichia coli	18	3	16.67	15	83.33	15	83.33	16	88.89	18	100	–	0	18	100	18	100	15	83.33	16	88.89	18	100	15	83.33	–	0	14	77.78	16	88.89
Citrobacter freundii	6	3	50	4	66.67	4	66.67	6	100	6	100	–	0	5	83.33	5	83.33	2	33.33	5	83.33	5	83.33	5	83.33	–	0	5	83.33	5	83.33
Proteus mirabilis	3	–	0	3	100	2	66.66	1	33.33	2	66.66	–	0	2	66.66	3	100	2	66.66	3	100	3	100	2	66.66	–	0	1	33.33	1	33.33
Pseudomonas aeruginosa	3	1	33.33	–	0	–	0	1	33.33	3	100	–	0	3	100	3	100	1	33.33	1	33.33	1	33.33	1	33.33	–	0	2	66.66	1	33.33
Klebsiella peumoniae	2	2	100	2	100	2	100	2	100	2	100	–	0	2	100	2	100	1	50	2	100	2	100	2	100	–	0	2	100	2	100
Shigella species	2	2	100	2	100	2	100	2	100	2	100	–	0	2	100	2	100	2	100	2	100	2	100	2	100	–	0	2	100	2	100
Providencia alcalifaciens	1	–	0	1	100	1	100	1	100	1	100	–	0	1	100	1	100	1	100	1	100	1	100	1	100	–	0	1	100	1	100

AML :Amoxycillin
AMP : Ampicillin
AMC : Amoxycillin/Clavulanic acid
C : Chloramphenicol
E : Erythromycin
CIP : Ciprofloxacin
OB : Cloxacillin
DNF : Danofloxacin
ENR : Enroflorxacin
S : Streptomycin
UB : Flumequine
CN :Gentamicin
NA : Nalidixic acid
P : Penicillin
TE : Tetracyciline

Table (7) : Number and percentage of resistant bacteria recovered from Arabian mares and aborted feti :

Antibacterial agent / Organism	No. tested	AML		AMC		AMP		C		CIP		OB		DNF		ENR		E		UB		CN		NA		P		S		TE	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Escherichia coli	18	17	94.4	1	5.6	1	5.6	1	5.6	–	0	18	100	–	0	–	0	1	5.6	1	5.6	–	0	1	5.6	18	100	4	22.22	1	5.6
Citrobacter freundii	6	5	83	1	16.7	1	16.7	–	0	–	0	6	100	1	16.7	1	16.7	4	66.7	1	16.7	1	16.7	1	16.7	6	100	1	16.7	1	16.7
Proteus mirabilis	3	2	66	–	0	1	33.3	2	66.66	1	33.3	3	100	1	33.3	–	0	1	33.3	–	0	–	0	1	33.3	3	100	2	66.66	2	66.66
Pseudomonas aeruginosa	3	2	66.66	1	100	3	100	2	66.66	–	0	3	100	–	0	–	0	2	66.66	2	66.66	2	66.66	2	66.66	3	100	1	33.33	2	66.66
Klebsiella pneumoniae	2	–	0	–	0	–	0	–	0	–	0	2	100	–	0	–	0	1	50	–	0	–	0	–	0	2	100	–	0	–	0
Shigella species	2	–	0	–	0	–	0	–	0	–	0	2	100	–	0	–	0	–	0	–	0	–	0	–	0	2	100	–	0	–	0
Providencia alcalifaciens	1	1	100	–	0	–	0	–	0	–	0	1	100	–	0	–	0	–	0	–	0	–	0	–	0	1	100	–	0	–	0

AML :Amoxycillin AMP : Ampicillin AMC : Amoxycillin/Clavulanic acid C : Chloramphenicol E : Erythromycin

CIP : Ciprofloxacin OB : Cloxacillin DNF : Danofloxacin ENR : Enroflorxacin S : Streptomycin

UB : Flumequine CN :Gentamicin NA : Nalidixic acid P : Penicillin TE : Tetracycline

DISCUSSION

Gynaecological diseases like abortion, infertility, endometritis, etc, are the most feared diseases in any equine breeding establishment. The etiological factors associated with equine gynaecological diseases comprise a wide variety of bacteria belonging to families *Enterobacteriaceae* and and many other families.

As shown in Table (3) the bacteriological examination of 167 diseased Arabian mares and 4 aborted feti revealed the isolation of Gram negative bacteria from 128 cases (74.85%), In this concern, McCue et al. (1991); Uppal et al. (1994) and Moreno et al. (1995) recovered Gram negative bacteria from diseased mares in incidences of 40%, 23.5% and 40% , respectively.

E. coli was recovered from cases of endometritis, pyometra and repeat breeder mares as well as aborted feti. This finding is in agreement with Hatem et al. (1989, b) and Winiarczyk et al. (2002) who isolated *E. coli* from cases of abortion. The relatively high incidence of recovery of E. coli may reflect the importance of *E. coli* as the cause of abortion and other gynaecological affections in mares as well as mortalities in neonatal foals (Hatem et al., 1989,b). Others considered *E.coli* as a significant cause of abortion in mares as Silva et al. (1999) and Schiemann et al. (2001). Moreover, Blobel et al. (1987) isolated *E.coli* from cases of infertile mares .

C. freundii was isolated in incidence of (4.68%) which is nearly similar to the record of Garg and Manchanda (1986) as they record incidence of (4.57%) .

C. freundii was isolated from mares suffering from endometritis, pyometra and repeat breeder. This finding is in agreement with that of Sharma et al. (1986) who recorded *C. freundii* as one of aerobic bacteria isolated from uteri of brood mares.

In the present investigation, *P. mirabilis* was isolated from diseased cases. O'Driscoll (1977) found that the route of infection could be through vaginal contamination with fecal material or through coitus.

In the present study *P. mirabilis* was isolated from repeat breeders, endometritis and pyometra in Arabian mares, which agree with Vural et al. (1999) who isolated *Proteus* from mares with endometritis and Joseph and Devendran (1987) who isolated *Proteus* from infertile thoroughbred mares.

Sharma et al. (1986) and MacLeay and Kohen (1988) suggested that Proteus species represented one of the normal bacterial flora of equine urogenital tract, the results obtained in the present study agree with this opinion that when the immunity of mares decrease, this microorganism become activated and cause disease .

On the other hand, as shown in Table (2), the recovery of *P. aeruginosa* from 3 out of 19 aborted mares (15.79%) as well as one out of 21 endometric mares (4.76%) is probably the most important finding in the present investigation which reflects the important role of this bacteria as a possible cause of abortion in mares.

The importance of *P. aeruginosa* is increasingly realized in the world, so several authors documented the significance of this organism as an equine pathogen where *P. aeruginosa* was isolated from cases of abortion and aborted equine feti by Garg and Manchanda (1986) with percentage of 10%; El Baroudy (1987) and Hatem et al. (1989,b) with percentage of 25% each. This reflecting the possible role played by this organism in equine abortion and fetal death. This is in agreement with the present study in which *P. aeruginosa* represent 15.79% of causes of abortion in mares.

Moreover, Anzai et al. (1991) and Moreno et al. (1995) recovered *P. aeruginosa* from cases of metritis. This agrees with the present study where *P. aeruginosa* was recovered from cases of endometritis.

The results obtained from this study (Table 2) revealed that, *E. coli* 16(84.21%), *P. aeruginosa*, 3 (15.79%), Streptococcus spp. 2(10.53%) and Staphylococcus spp. were the most common bacterial isolates recovered from cases of abortion. This finding is in agreement with Garg and Manchanda (1986); El Baroudy (1987) and Hatem et al. (1989,b).

The maximum abortions rate occurred in Arabian mares within 4 years of investigation in El-Zahraa Stud and private studs was recorded in winter season (December- February) with an average of (2.98%) and (3.06%); in autumn season (September - November) with an average of (2.71%) and (2.04%) respectively as shown in Tables (4 & 5) which is in agreement with Garg and Manchanda (1986) who recorded maximum abortions during winter season.

In the present investigation, the antibiogram study of *E. coli* revealed 100% sensitivity to ciprofloxacin, danofloxacin, enrofloxacin and gentamicin, the obtained results of gentamicin are similar to those reported by Hatem et al. (1989, b) and Moreno et al. (1995).

The data on the antibiograms presented in Table (6) show that *C. freundii* were highly sensitive to chloramphenicol (100%), ciprofloxacin (100%), followed by danofloxacin, enrofloxacin, flumequine, gentamicin, nalidixic acid, streptomycin and tetracycline (83.33% each). In this concern, Sharma et al. (1986) recorded that Gram negative bacteria isolated from brood mares were highly sensitive to chloramphenicol and gentamicin than to tetracycline, streptomycin and erythromycin.

In the present investigation, the antibiogram

study on the recovered *P. mirabilis* indicated that ampicillin, flumequine and gentamicin were the most prominent effective against it. This is in agreement with Silva et al. (1999). On the other hand El Baroudy (1987) found that *P. mirabilis* was sensitive at rates of 80%, 100% 40% and 50%, respectively against chloramphenicol, flumequine, gentamicin and tetracycline, and resistant against ampicillin. The resistance to ampicillin may be due to the fact that this antibiotic was extensively used in veterinary practice in Egypt during the past 20 years.

In the present investigation, the antibiogram study of the recovered *P. aeruginosa* revealed that the highly effective drugs against it were ciprofloxacin, danofloxacin and enrofloxacin with 100% susceptibility. On the other hand, Hatem et al. (1989, a), Moreno et al. (1995) and Silva et al. (1999) found that gentamicin was the best antibiotic against *P. aeruginosa*. Ciprofloxacin and enrofloxacin are relatively a new chemotherapeutic agents which may explain the high susceptibility of Pseudomonas isolates to them. The multiresistance property of *P. aeruginosa* may be attributed to physicochemical properties of the cell wall rather than the antibiotic inhibitory enzymes (Koncicki and Szubstarska, 1988 and Ezz-Elden, 1996).

The antibiogram study of the isolated *K. pneumoniae* showed that it was highly sensitive to gentamicin and nalidixic acid. This finding is in agreement with Tainturier and Richard (1986), El - Seedy et al. (1991) and Ozgur et al. (2003). In addition, they found that *K. pneumoniae* was intermediately sensitive to chloramphenicol and streptomycin, while in the present study it was 100% sensitive, this may be due to the fact that these antibiotics are not commonly used now in veterinary practice in Egypt.

The results of antibiogram study carried out on the two shigella isolates, as shown in Table (6) revealed that both of them were sensitive to all tested antibiotics except cloxacillin and penicillin. This sensitivity may be due to the fact that these drugs are not commonly used for the treatment of shigella infection in mares in Egypt.

The data on the antibiogram presented in Table (6) showed that *Providencia alcalifaciens* was highly sensitive to amoxycillin/ clavulanic acid, ampicillin, chloramphenicol, ciprofloxacin, danofloxacin, enrofloxacin, erythromycin, flumequine, gentamicin, nalidixic acid, streptomycin and tetracycline (100% each).

Depending on the obtained results the following points are recommended:

1-Routine bacteriological examination for mares before matting to detect any reproductive problem.

2- using bacterial-free healthy stallions for matting.

3- In case of incrimination of bacterial pathogens,

it is highly recommended to make sensitivity test as soon as possible to be able to give the proper antimicrobial drug to ensure proper treatment and to avoid the emergence and existence of drug resistant bacteria and failure of treatment (which is usually very expensive).

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