Highlighting on the Abortion Syndrome in Arabic Mares

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***Abstract*—** Abortion in horses may consequence from a multiplicity of causes. An infectious agent, as bacteria, viruses or fungi is one of these causes, which outcome the fetus or its tissues, causing fetal death and expulsion. A total 126 isolates (94.7%) was from 361 samples. Aborted feti showed the highest rate of pathogens (43.6%) followed by uterine wash 34 (25.6%); vaginal washes 30 (22.6%) and the lowest rate was from semen samples 4 (3.0%). *K.pneumoneae* showed the highest rate 15.0% of pathogenic strains. Ciprofloxacin is the best drug of choice for most isolated pathogens. The prime identification of opportunistic bacteria advises the need of successful hygiene management of breeding Arabian mares to inhibit bacterial infection that may be the origin of fetal loss, stillbirth, and perinatal mortality. MDR bacteria with risky resistance against antibiotics used in equine and have been being a probable public health hazard in Arabian equine in Egypt were reported..

***Keywords*—** B. cepacia complex Equine abortion, K. pneumoneae, Multidrug Resistanse

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I. INTRODUCTION

Abortion occurs in a range of 10–15% of equine pregnancies and may have infectious and non-infectious causes. Infections with viruses, fungi or bacteria cause inflammation to the placenta and affect the placental function. The most common bacterial causes of equine abortion include *Streptococcus zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Leptospira sp*. and *Nocardia sp*. Several other bacterial species have been implicated as cause of equine abortion but occur less commonly [1].

Different aerobic microbes can reach the uterus during mating, artificial conception, reproductive inspection or parturition. Infections are mainly caused by opportunistic or commensal microorganisms, such as *Streptococcus zooepidemicus, Escherichia coli* and *Staphylococcus aureus*. Other microorganisms like *Taylorella equigenitalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and leptospira are transmitted through venereal route [2].

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II. MATERIAL AND METHODS

A total number of 360 samples collected, from 38 aborted feti (265) internal organs and placenta); 55 mares (where 29 uterine washes and 26 vaginal washes) and 40 stallions (40 semen samples).

All samples were inoculated Buffer peptone water (BPW) and then subcultured on blood agar, macCokey agar, and specific media of each suspected microorganism as mannitol agar, tinsidal media, Alloa media pseudomonas specific media, eosin methelyne media. In case of campylobacter isolation, the samples were inoculated on thioglycollate broth as enrichment media then subcultured on campylobacter specific media and each plate incubated at suitable temperature and duration. In case of salmonella isolation, 0.1 ml of cultured BPW was inoculated on Rappaport Vassiliadis broth (RV) for 18h at 37C then subcultured on Xylose Lysine Deoxycholate media (XLD) and each plate incubated at suitable temperature and duration [3,4,5 6,7]. The suspected colonies were subjected to further identification biochemically and by using SRO GP24 and SRO GN24 (Diagnostics i.n.c.). Clinical isolates (126) were submitted for antimicrobial susceptibility test using different antibiotics recorded in Table (1) and detection of extended-spectrum-β-lactamase (ESBL)-producing bacteria using E-test technique based on ESBL E-test®[8]. Cefotaxime 0.002-32mg/l (CTX), Cefotaxime 0.016-256mg/l (CTX), Cefepime/Cefepime Clavulanic acid 0.25-16/0.064-4mg/l (FEP/FEL), Amoxicillin 0.016-256 mg/l (AML), Cefotaxime/Cefotaxime +Clavulanic acid 0.25-16/0.16-1 mg/l (CTX/CTL), Ampicillin-Sulbactam, 0.016-256mg/l (AMS) Ciprofloxacin 0.002-32 mg/l (CIP) (Liofilchem)

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III. Results and Discussion

A total 126 isolates (94.7%) was from 360 samples represent 133 animals. Aborted feti showed the highest rate of pathogens 58 (43.6%) Table (2) & Fig. (1). and the lowest rate was from semen samples 4 (3%).

In Gram positive bacteria, the highest pathogen isolated was *L.monocytogees* (19) *S.zooepidemicus* (11); Table (1) Fig. (2). [10;11;12]

In Gram negative bacteria, *K.pneumoneae* showed the highest number. The lowest isolated microorganisms were S.*Typhimurium; H.alveae and E. aerugenes* (1 for each) Tale (1) and Fig.(2). *K.pneumoneae* and *P.aeruginosa* were recorded as the most Gram negative isolates from the equine uterus [13,14,15] *K.pneumoneae* was isolated in a rate 7.1% from a perinatal death in horses diagnosed from 2000 to 2015 [16].

One isolate of *P.aeruginosa* was isolated from semen and four from uterine wash as well as 2 isolates of *K. pneumoneae* were isolated from uterine wash samples and 1 isolates from semen, these results clarified that these organisms are often introduced during coitus, insemination with infected semen, or genital manipulations.

By using Pearson correlation coefficient analysis, the nearly strong positive strength between Gram negative bacteria isolates from semen with that obtained from vaginal samples, aborted feti and uterine wash (0.97; 0.95; 0.93 respectively. which revealed the role of venereal infection with Gram negative bacteria and systemic infection [9]. While in Gram positive bacteria the negative relation between uterus washing and aborted feti (-0.10) and positive relation between vaginal and aborted feti isolates (0.62) as well as bacteria isolated from uterus and vaginal isolates (0.57) and no relation between bacteria isolated from uterus and semen and between bacteria isolated from vaginal and semen (r=0). These results clarified that most Gram positive bacteria causing abortion through environmental infection (Figures 3&4).' The rate of *L.monocytogenes* was 14.3% % (Table 2 & Fig. 5). *L.monocytogenes* is most commonly associated with encephalitis, septicemia, and abortion in veterinary species [17], however, clinical disease in the horse is rare. Previous reports of disease caused by this organism in horses ranging from 6 days to 6 years old include multi systemic infections [18]. The presence of *L. monocytogenes* may be due to feeding animals on silage which has a well-known risk factor for listeriosis, especially when used the silage of poor quality (pH > 5.5) [19,20].

Salmonella Arizona and *S. Typhimurium* were isolated in rate 3.8% and 0.8% respectively[21,22] *S. enterica* subsp. *arizonae* have not previously reported as a cause of abortion in pregnant mares and uncommonly isolated from equids in the literature, it may cause late-term abortions in susceptible animals [23]. In literature, the major causes of abortion, stillbirth, and perinatal mortality in regions of Brazil are Escherichia coli, *Enterobacter aerogenes*, *E. coli* and Streptococcus spp. and Staphylococcus spp. and Bacillus spp. Arcanobacterium pyogenes, Streptococcus spp.,Corynebacterium spp.,and *Rhodococcus equi*. [24,25, 26.27]. In the present study, *Burkholderia cepacia complex Bcc* were isolated in a rate of 9%. *Bcc was* isolated from uterine swabs of horses and cattle [28]. its distribution in animal species are not widely documented [29]. *E.coli* was isolated in a rate 3% *E. coli* are able to cause acute infection with bacteremia during early and mid-gestation in foals [25, 27]. Table (2) and Fig. (5).

Burkholderia cepacia complex Bcc were isolated in a rate of 9%. *Bcc was* isolated from uterine swabs of horses and cattle [28]. its distribution in animal species and associated infections are not widely documented [29]. It was found that *E. coli* are able to cause acute infection with bacteremia during early and mid-gestation in foals [25, 27]. *P.mirablis* was isolated in a rate of 3.8%, It was recovered from uterine diseased mares [30]. *S.zooepidemicus w*as isolated in a rate of 8.3% which is the most frequently isolated pathogen from the uterus of the mare [31], while *S. equi* was isolated in a rate of 6.9% which reported to be isolated from the equine endometrium [32,33]. Both *R.equi* and *S.aureus* were isolated in the same rate 3%, *A.haemolyticum* was isolated in a rate of 1.5 % from aborted mares (vaginal wash) [24]. Table (2) and Fig. (5)

A.haemolyticum was causing nasopharynx and skin infections in humans, it was characterized phenotypically and genotypically from seven *A. haemolyticum* six horses [34]. *H. alvei* (0.8%) was isolated from uterine wash of mares (Tables 2,3 & Fig. 3). [35]. In mares, *Hafnia alvei* (*H. alvei)* can cause abortions in different periods of gestation. In 1962, *H. alvei* was isolated in pure culture from the fetus and placenta. [36]

Only 11 isolates (55%) of *K. pneumonia* were sensitive to enrofloxacin followed cefotaxeme, cifoperazon and amikacin (40%, 35%, 35% respectively, Eight *P.aeruginosa* isolates were only sensitive to pipracillin-tazablam (66.7%). All BCC isolates were sensitive to Pipracillin-tazablam (100%) and Ciprofloxacin (33.3%), No systematic differences in antibiotic susceptibility have been noted across the different species of the complex. However, *B. cepacia complex* bacteria possess high-level resistance to many antibiotics such as the aminoglycosides, beta-lactams [37].

All isolates of *L.monocytogenes* were sensitive to Cifoperazon, azithromycin and ciprofloxacin (100%) followed by enrofloxacin 88.2% ampicillin (79%) and kanamycin 47.4%. *S.equi* isolates were sensitive to Pipracillin-tazabactam, Cifoperazon, Cifoperazon, Azithromycin enrofloxacin and Linezolid (100%), While *S.zooepidemicus* were sensitive only to ciprofloxacin 66.7%. *R. equi* were sensitive to Pipracillin-tazabactam , Cefquinome, Enrofloxacin, ciprofloxacin (100%). *E. Facium* sensitive to ampicillin, ampicillin-subactam and oxytetracyclin, these results revealed there is a need of rapid microbiological diagnosis so that suitable treatment of the infection can be achieved while the mare is still in oestrus. [32].

Several startling reports released by international organizations have defined the significances of this emergency at health and economic levels in animals and human. Heatmap analysis illustrated the intensity of antibiotic resistance of different isolates depending on the percentage of resistance (Figure 6). E-test strips with gradient concentrations of cefotaxime, ceftazidime, and cefepime at one end and

cefotaxime, ceftazidime, or cefepime with clavulanic at the other end were used All isolates shows 100% positive ESBL with CTX at two concentration except *K.pneumoneae*, for CTX and FEP/FEL (Tables 4,5&6). *P.aeruginosa*, salmonella species, *S.zooepidemicus* and *L.monocytogenes*, showed 100% positive ESBL with all MIC strips except CIP (Tables 3,4&6). All *E.coli* and *S.aureus* isolates were 100% positive to all ESBL strips (Tables 3,4). *S.equi* and *BCC* 100% positive to all ESBL strips except AMS and CIP strips (Tables 4&5) . Detection of Gram negative Bacteria and ESBL Gram positive accelerates clinical inference and infection control measures and finally give improve treatment for infected animals.

Equally important, the rates of Gram-positive resistant isolate resistance to antibiotics are also forceful the success of treating infectious diseases. *Staphylococcus aureus* and other beta-lactamase-producing pathogens such as *S. zooepidrmicus*, *S. equi L.moocyeogenes*, and *R.equi* are among the troublemaker). It was reported a higher proportion of beta-lactamase, and particularly ESBL-producing pathogens with resistance to β-lactam antibiotics among Gram-negative and Gram-positive bacteria [38,39].. Chromosomal AmpC b-lactamase can interfere with clavulanate synergy from a co-existing ESBL [40].

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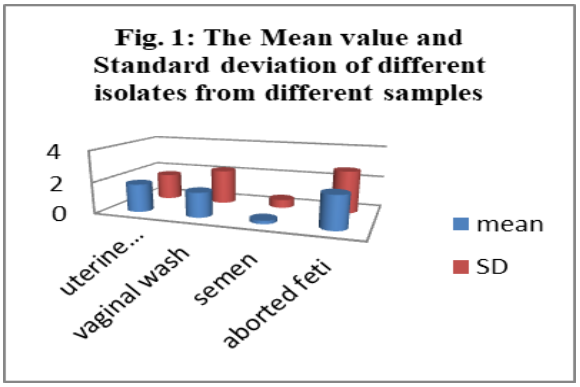


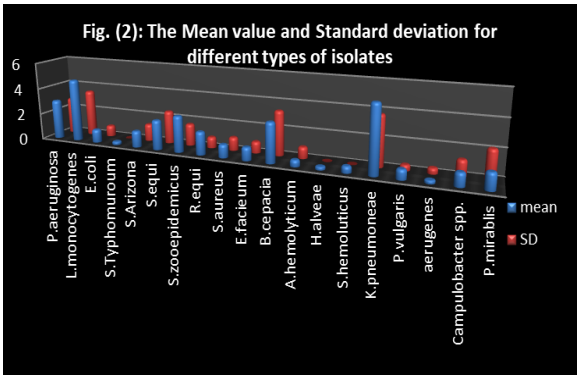


TABLE I:

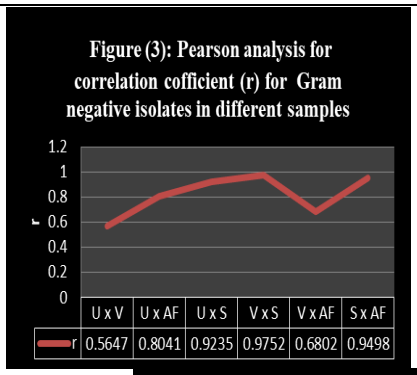
Number &percent of different pathogenic isolates from mares, stallions and aborted feti

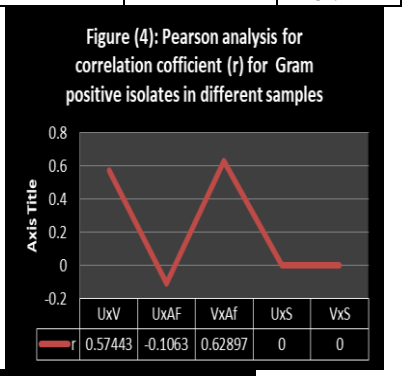
Type of pathogens	Type of animals (133)										
	Mares (55				Stallions (40)		Aborted feti (38)		Total	Mean	S D
	Uterine wash (29)		Vaginal wash (26)		Semen (40)		Internal organs (266)				
	No	%*	No	%	No	%	No	%			
P.aeruginosa	**4**	**3**	**0**	**0**	**1**	**0.75**	**7**	**5.26**	**12**	**3**	**2.77**
L.monocytogenes	**5**	**3.75**	**7**	**5.26**	**0**	**0**	**7**	**5.26**	**19**	**4.75**	**3.5**
E.coli	**1**	**0.75**	**1**	**0.75**	**0**	**0**	**2**	**1.5**	**4**	**1**	**0.83**
S.Typhimurium	**1**	**0.75**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.25**	**0**
S.Arizona	**2**	**1.5**	**0**	**0**	**0**	**0**	**3**	**2.25**	**5**	**1.25**	**1.29**
S.equi	**2**	**1.5**	**1**	**0.75**	**0**	**0**	**6**	**4.51**	**9**	**2.25**	**2.48**
S.zooepidemicus	**5**	**3.75**	**2**	**1.5**	**0**	**0**	**4**	**3**	**11**	**2.75**	**1.65**
R.equi	**4**	**3.**	**1**	**0.75**	**0**	**0**	**2**	**1.5**	**7**	**1.75**	**0.82**
S.aureus	**0**	**0**	**2**	**1.5**	**0**	**0**	**2**	**1.5**	**4**	**1**	**1**
E.facieum	**0**	**0**	**0**	**0**	**2**	**1.5**	**2**	**1.5**	**4**	**1**	**0.86**
B.cepacia	**3**	**2.25**	**1**	**0.75**	**0**	**0**	**8**	**6.01**	**12**	**3**	**3.34**
A.hemolyticum	**0**	**0**	**2**	**1.5**	**0**	**0**	**0**	**0**	**2**	**0.5**	**0.86**
H.alveae	**1**	**0.75**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0.25**	**0**
S.hemoluticus	**2**	**1.5**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0.5**	**0**
K.pneumoneae	**2**	**1.5**	**8**	**6.01**	**1**	**0.75**	**9**	**6.76**	**20**	**5**	**3.76**
P.vulgaris	**2**	**1.50**	**1**	**0.75**	**0**	**0**	**0**	**0**	**3**	**0.75**	**0.43**
E.aerugenes	**0**	**0**	**1**	**0.75**	**0**	**0**	**0**	**0**	**1**	**0.25**	**0.43**
Campulobacter spp.	**0**	**0**	**3**	**2.25**	**0**	**0**	**1**	**0.75**	**4**	**1**	**1.22**
P.mirablis	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**3.75**	**5**	**1.25**	**2.16**
Total No. &% of isolates in each type of samples	**34**	**25.6**	**30**	**22.6**	**4**	**3**	**58**	**43.6**	**126**	**Negative samples**	
Mean	**1.8**	**1.3**	**1.6**	**1.18**	**0.2**	**0.15**	**3.1**	**2.29**	**%**	**No**	**%**
SD	**1.7**	**1.2**	**2.2**	**1.66**	**0.5**	**0.39**	**2.98**	**2.24**	**94.7**	**7**	**5.3**

*: percent is calculated according to the total No. of animals (133)

TABLE II
Rate of different isolated microorganisms among total No. of animals (133)

Type of Isolate	No. isolates	%
klebsiella pneumonia subspp. pneumoneae.	20	15.0
Listeria monocytogenes	19	14.3
***Pseudomonas** aeruginosa*	12	9.0
Burkholderia cepacia complex Bcc	12	9.0
Streptococcus equi subsp.zooepidemicus	11	8.3
Streptococcus equi subsp. Equi	9	6.8
Rhodococcus equi	7	5.3
Salmonella Arizoneae	5	3.8
P.mirablis	5	3.8
E.coli	4	3.0
Enterococcus faecium	4	3.0
Staphylococcus aureus	4	3.0
Campylobacter spp	4	3.0
P.vulgaris	3	2.3
A.hemolyticum	2	1.5
Staphylococcus haemolyticus	2	1.5
Salmonella Typhimurium	1	0.8
H.alveae	1	0.8
Enterococcus aerogenes	1	0.8
Total	126	94.7





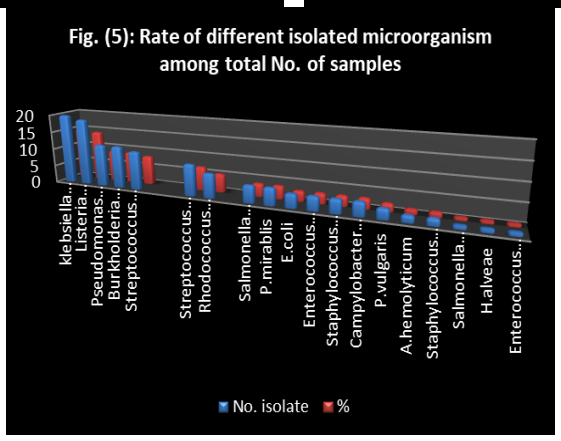


TABLE III: MIC strip ESBLs results [8&41]

Type of MIC strips*	Concentration mg/l	Type of isolated bacteria															
		P.aeruginosa (12)				*Klebsiella pneumoneae* (20)				*E.coli* (4)				*Salmonella species* (6)			
		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
CTX	0.002-32	12	100	0	0	17	85	3	15	4	100	0	0	6	100	0	0
CTX	0.016-256	12	100	0	0	12	60	8	40	4	100	0	0	6	100	0	0
FEP/FEL	0.25-16/0.064-4	12	100	0	0	19	95.5	1	5	4	100	0	0	6	100	0	0
AML	0.016-256	12	100	0	0	20	100	0	0	4	100	0	0	6	100	0	0
CTX/CTL	0.25-16/0.16-1	12	100	0	0	20	100	0	0	4	100	0	0	6	100	0	0
AMS	0.016-256	12	100	0	0	20	100	0	0	4	100	0	0	6	100	0	0
CIP	0.002-32	10	83.3	2	16.7	17	85	3	15	4	100	0	0	4	66.7	2	33.3

*CTX: Cefotaxime,. FEP/FEL: Cefepime/Cefepime+Clavulanic acid, AML: Amoxicillin, CTX/CTL: Cefotaxime/Cefotaxime+Clavulanic acid,,; AMS: Ampicillin-Sulbactam, CIP: Ciprofloxacin (Liofilchem)

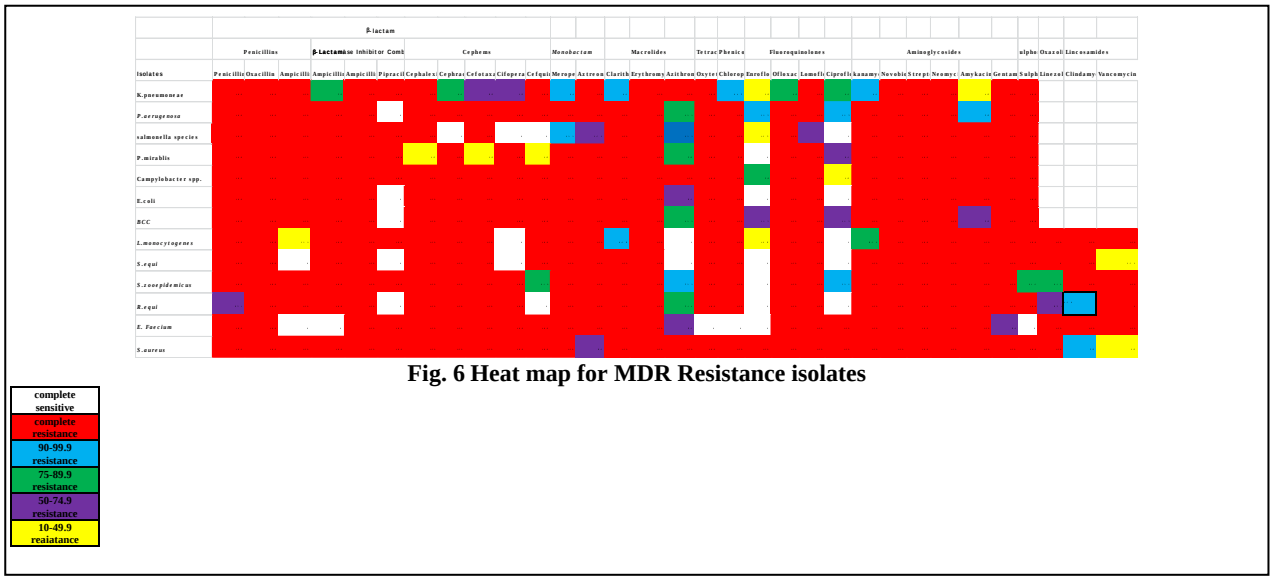


Fig. 6 Heat map for MDR Resistance isolates

TABLE IV: MIC strip ESBLs results [8]

Type of MIC strips*	Concentration mg/l	Type of isolated bacteria															
		S.zooepidemicus (11)				*Staphylococcus aureus* (4)				*L.monocytogenes* (19)				*S.equi* (9)			
		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
CTX	0.002-32	11	100	0	0	4	100	0	0	19	100	0	0	9	100	0	0
CTX	0.016-256	11	100	0	0	4	100	0	0	19	100	0	0	9	100	0	0
FEP/FEL	0.25-16/0.064-4	11	100	0	0	4	100	0	0	19	100	0	0	9	100	0	0
AML	0.016-256	11	100	0	0	4	100	0	0	19	100	0	0	9	100	0	0
CTX/CTL	0.25-16/0.16-1	11	100	0	0	4	100	0	0	11	100	0	0	9	100	0	0
AMS	0.016-256	11	100	0	0	4	100	0	0	11	100	0	0	8	88.9	1	11.1
CIP	0.002-32	5	45.5	6	54.5	4	100	0	33.3	1	5.3	18	94.7	6	66.7	3	33.3

***CTX: Cefotaxime,. FEP/FEL: Cefepime/Cefepime+Clavulanic acid, AML: Amoxicillin, CTX/CTL: Cefotaxime/Cefotaxime+Clavulanic acid,,; AMS: Ampicillin-Sulbactam, CIP: Ciprofloxacin (Liofilchem)**

TABLE V MIC strip ESBLs results [8&41]

Type of MIC strips*	Concentration mg/l	*BCC* (12)				*R.equi* (7)			
		+ve for ESBL		-ve for ESBL		+ve for ESBL		-ve for ESBL	
		No.	%	No.	%	No.	%	No.	%
CTX	0.002-32	12	100	0	0	7	100	0	0
CTX	0.016-256	12	100	0	0	7	100	0	0
FEP/FEL	0.25-16/0.064-4	12	100	0	0	7	100	0	0
AML	0.016-256	12	100	0	0	7	100	0	0
CTX/CTL	0.25-16/0.16-1	12	100	0	0	7	100	0	0
AMS	0.016-256	10	83.3	2	16.7	7	100	0	0
CIP	0.002-32	10	83.3	2	16.7	7	100	0	0

***CTX: Cefotaxime,.**

FEP/FEL: Cefepime/Cefepime+Clavulanic acid, AML:Amoxicillin, CTX/CTL: Cefotaxime/Cefotaxime+Clavulanic acid,,;

AMS: Ampicillin-Sulbactam, CIP: Ciprofloxacin (Liofilchem