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BACTERIAL ISOLATION AND IDENTIFICATION FROM ABORTED PLACENTA OF WOMEN AND EWES AND GOAT IN AL-FALLUJAH CITY

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Abstract

In order to determine the percentage of important zoonotic bacteria isolated from aborted placenta of women and small ruminant (ewes and she goat) in addition to evaluate the antibiotic resistance of one important bacteria isolates, to achieve these goal aborted placenta sample were collect aseptically during October/ 2018-May/2019 from Al- Fallujah city, these samples were culture on routine bacteriological media, bacterial identification were done by biochemical test, Rose Bengal test, Api 20 kits, were used and some suspected isolates were confirmed by Vitek® assay. The result of bacterial isolation showed among one hundred samples include 32 species of bacteria which were total positive isolated which indeed enclose 12(24%) of animal samples which were 50 sample and 20(40%) of human which were 50 sample include*: Serratia fonticola, Granulicta elegata, Ochromobacterium anthropic, Staphylococcus cohnii, Proteus mirabilis, Achromobacter xylosoxidans, Kocuria kristinae, Staphylococcus xylosus, Chromobacterium violaceum, Proteus vulgaris. Staphylococcus epidermidis, Citrobacter freundii, Pantoea* spp., *Enterococcus fecalis, Salmonella* spp., *Streptococcus* spp., *E. coli, Klebsiella pneumonia, Campylobacter jejuni, Bacillus* spp. *and Pseudomonas aeruginosa* isolates expressed resistant to Ciprofloxacin, Levofloxacin, Tobramycin, Impenem, Cefapime, Piperacillin + tazobactam and sensitive to ofloxacin, Polymyxin B, Amikacin and aztreonam. It was concluded that varies bacteria were isolated from aborted placenta of sheep and goat and *P. aeruginosa* form a second bacterial isolated, these bacteria expressed multiantibiotic resistance.

Key words: placenta, women, ewes, goat.

Introduction

The main source of meat and milk in Iraq is sheep and goats in which infertility was influence on their production, abortion is considered essential problem associated with animal fertility particularly abortion occur by wide range of factors include, mechanical, nutritional,chemical factors in addition to biological agents such as bacteria and fungi,parasite and viruses (Sargison *et al*., 2001). In addition to contagious disease, such as *Brucella melitensis, Listeria monocytogenes, Escherichia coli, Campylobacter, Salmonella* spp., *Staph. aureus, Leptospira, Streptococci, Chlamydia* spp. and *Corynebacterium pyogenes* (Bajmocy *et al*., 1987). High incidence of abortion occur in sheep and goats induced by Campylobacter fetus that may reach up to 70% (Bajmocy *et al*., 1987). The major opportunistic pathogen of humans and animals infection is *P. aeruginosa* particularly those suffering from impaired immune response (Ruiz-Roldán *et al*., 2018), *P. aeruginosa* can cause enzootic or epizootic outbreaks mastitis in the small ruminant (Sela *et al*., 2007). *Pseudomonas aeruginosa* possess a wide arsenal virulence factors, therefore, it can induced both acute and chronic infection in wide range of hosts include humans and animals (Driscoll *et al*., 2007) and these pathogen can resistant host defense mechanisms and repeated antibiotic treatment (Sadikot *et al*., 2005). These pathogen can resistant antipseudomonal penicillins, fluoroquinolones, cephalosporins, aminoglycosides and carbapenems (Odumosu *et al*., 2016) also (Ruiz-Roldán *et al.*, 2018) recorded that 72 *P. aeruginosa* isolated from 1,443 faecal samples and they found that these strains expressed low antimicrobial resistance levels such as ceftazidime (8%), gentamicin (3%), cefepime (7%), ciprofloxacin (1%), aztreonam (7%) and imipenem (1%); susceptibility to amikacin, levofloxacin, meropenem, tobramycin and colistin serious therapeutic problem occur in the treatment of *P. aeruginosa* in the clinical cases due to wrong using of antibiotic drugs (Peng *et al*., 2014).

Materials and Methods

All media were prepared according to the manufacturer's instructions and sterilized by autoclave at 121°C and 15 psi for 15 minutes.

Sample collection

One hundred aborted placental samples of aborted women and small ruminant (ewes and she goat), fifty for each one, were collected from Teaching Hospital for Women and Children in Fallujah city took biopsy from curettage operation and from Veterinary hospital and clinical veterinary (Governmental institution and Private clinic), then transferred to the laboratory by sterile container inside cool box to trituration the placenta sample in mortar after heating spatula on the surface of sample to sterilized then cut by scalpel to take from inside a sample and mix with PBS inside a clean and sterile mortar to make a homogenized mixture then cultured aerobically for 24 hours and anaerobically for 72 hours on blood agar, MacConkey agar and nutrient agar then put in incubation at 37°C after check the incubation free from contamination by put un-inoculated plate of BHI agar for 24hours before put the isolated in the next day transferred to selective media.

Results

The result of bacterial isolation showed among one hundred samples include 32 species of bacteria which were total positive isolated which indeed enclose 12(24%) of animal samples which were 50 sample and 20(40%) of human which were 50 sample that reveal in table 1.

Isolation from women

The result of 20 positive species from 50 sample of women which equal 40% from total positive number which reveal in table 2.

The result of 12 positive species from 50 sample small ruminant (sheep and goat) which equal 24% from total positive number which reveal in (Table 3).

Pseudomonas aeruginosa

Choose isolation of *P. aeruginosa* which was most frequent and considered as highly pathogenic zoonotic causative agent in abortion which reveal 62.5% this consider the highest percentage among isolated bacteria, so; selected of *P. aeruginosa* for deep research.

Table 1: The number of placenta sample collected from animal and women, and positive isolated of Micro-Organism and percentage % of them.

Percentage % from total positive no. (32)	Positive isolation of bacterial species	Number of sample	Source of placenta sample
24%	12	50	Animal small ruminant (ewes and she goat)
40%	20	50	Women

Table 2: The number and percentage of bacterial species isolated from placenta of aborted women.

Percentage %	Positive no. for each Spp. from 20 positive isolate	Bacteria species
20	4	*Staphylococcus aureus*
5	1	*Serratia fonticola*
10	2	*Salmonella* spp.
10	2	*Citrobacter freundii*
5	1	*Granulicatella* adiacens
15	3	*Staphylococcus epidermidis*
5	1	*Ochromobacterium anthropic*
75	15	*Pseudomonas aeruginosa*
30	6	*Klebsiella pneumonia*
5	1	*Staphylococcus cohnii*
5	1	*Citrobacter youngae*
5	1	*Chromobacterium violaceum*
5	1	*Achromobacter xylosoxidans*
5	1	*Kocuria kristinae*
10	2	*Pantoea* spp.
10	2	*Enterococcus fecalis*
5	1	*Staphylococcus xylosus*
10	2	*Streptococcus* spp.
10	2	*E. coli*
5	1	*Proteus vulgaris*

Pseudomonas aeruginosa Cultural characteristics of

On MacConkey's agar the colonies appeared pale

Table 3: The number and percentage of bacterial species isolated from aborted placenta of small ruminant.

Percentage %	Positive no. for each Spp. from 20 positive isolate	Bacteria species
50 %	6	*Staphylococcus aureus*
33.3 %	4	*Staphylococcus epidermidis*
33.3 %	4	*Citrobacter freundii*
50 %	6	*Klebsiella pneumonia*
33.3 %	5	*E. Coli*
33.3 %	4	*Pantoea* spp.
41.6 %	5	*Pseudomonas aeruginosa*
33.3 %	4	*Salmonella* spp.
16.6 %	2	*Achromobacter xylosoxidans*
25 %	3	*Campylobacter jejuni*
25 %	3	*Bacillus* spp.
33.3 %	4	*Streptococcus* spp.

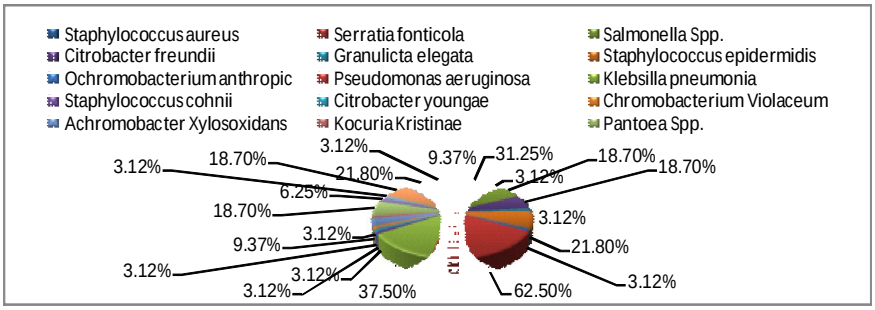

Fig. 1: Pie chart shows the percentages of bacterial which isolated from total aborted placenta.

and non-lactose fermenter, on Blood agar, the colonies were large, irregular in shape, grey in color and surrounded by a zone of ß-hemolysis, on selective media such as Pseudomonas agar base revealed fastly bacterial growth and produced pigments so appeared blue-green in color and when used Cetrimide agar base, the isolates appear a characteristic blue-green to yellow-green color.

Isolates was identification by using of the API 20E

• Identification by used VITEK®:

Vitek2 system used to confirm the diagnosis *Pseudomonas aeruginosa* based on the results of 64 biochemical tests and 99% probability value as shown in fig. 2.

Discussion

The current study revealed numerous bacterial isolates from aborted placenta of small ruminants and women, in addition all these isolates are opportunistic bacteria, these result may indicated that opportunistic pathogen may play a role in abortion in women and small ruminant these idea was agreement with Macpherson, (2005). Who recorded that opportunistic bacteria are form the majority of placental infection that migrated from caudal reproductive tract to uterus, also the present finding may indicated that these opportunistic pathogen cause uterus infection as a result un-appropriated condition of the small animal genital tract that lead to abortion.

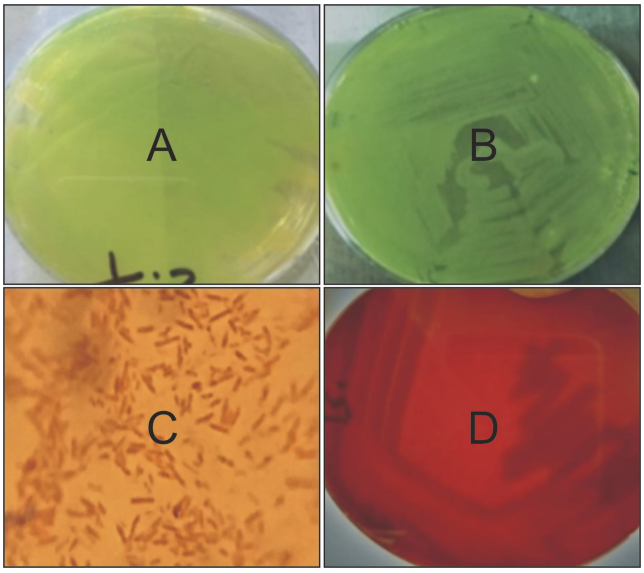

Fig. 2: (A) Appear *P.aeroginosa* on cetrimide agar base; (B) *P. aeroginosa* on Pseudomonas agar base; (C) ß-hemolysis of *P.aeroginosa* on blood agar; (D) Under the microscope stained by Gram stain (X100).

The opportunistic pathogens can inhabitants the environment and the host and they are able enter the blood circulation and reach the reproductive organ and induced abortion. (Holler, 2013), also Sara *et al.*, (2017) isolated certain opportunistic bacteria from bovine aborted placenta include *Streptococcus spp, E. coli* and *Pseudomonas*; however, placenta may exposed to several contaminated agents in which these agent may be not found in the internal fetal organs. (Hopper, 2015).

The current study showed that *Staph. aureus* 6 (50%) and *K. pneumoniae* 6(50%) form high percentage of bacterial isolates from aborted placenta of animals followed by *P. aeruginosa* and *E. coli* (41.6% for each one), these result may give indication that these pathogen play role in ovine abortion, these result agreement with Bizzarro *et al.*, (2013) who revealed that the Pseudomonas form the most bacterial isolated from bovine aborted placental samples.

The isolation of several bacteria from aborted placenta of small ruminant may indicated that these bacteria can reach the uterus from other part of genital tract, in Iraq, Al-Hilla, Al-Zubaidi, (2015) isolated *Escherichia coli* (28.97)%, *Klebsiella* spp. (16.82)%, *Salmonella* spp. (14.95)%, *Proteus* spp. (13.08)%, *Staphylococcus aureus* (11.21)%, *Staphylococcus epidermidis* (8.41)% and *Streptococcus* spp. (6.54)%. from genital tract of cattle, also (Burfeind *et al.*, 2014). Isolated several bacteria from bovine uterus include *Arcanobacterium pyogenes, Escherichia coli, Clostridium perfringens, Corynebacterium* spp., *Staphylococcus aureus, Streptococcus uberis, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia and Bacillus* spp.,

The isolated of *Pseudomonas aeruginosa* and *Klebsiella, E. coli* from aborted placenta in the present study was in consistent with result of Giles *et al.*, (1993). Who isolated *Escherichia coli, Pseudomonas aeruginosa, Streptococcus equi* subsp. *zooepidemicus* and *Klebsiella pneumoniae* from aborted placenta in mare in addition, Hines (2007). Isolated *Klebsiella* spp. from aborted placenta of mares, the isolated large number of bacteria

Fig. 3: API 20E results for *Pseudomonas aeruginosa.*

from aborted placenta of small ruminant may indicated that the abortion may induced by bacterial infections in the present result.

Pereira *et al.,* (2014) reported that bacterial infection form 36.1% of etiology of abortion in mare and *K. pneumoniae* was isolated from 5.6% of the mare fetuses also In, Gressler *et al*., (2014) recorded that *Klebsiella* experimentally induced placentitis and abortion in mares.

The isolated of *Campylobacter jejuni* from aborted placenta of small ruminant may give indicator that these pathogen was considered one cause of abortion in small ruminant these idea was agreed with West, (2002) who found that these bacteria cause bacterial abortion storms and stillbirth in sheep in UK and New Zealand also, as well as Lacasta *et al.,* (2015). Found that all isolates of *C. jejuni* from ovine abortion expressed resistant for cycline *C. jejuni* form the 4th bacterial isolated from small ruminant placenta in the present study.

The isolated food borne pathogens such as *P. aeruginosa, Salmonella, Staph. aureus* and *C. jejuni* from aborted placenta of small ruminant may give indicated that the zoonotic bacteria are responsible for animal abortion, these idea was agreement with Roest *et al.,* (2011) who isolated zoonotic pathogens from aborted dairy goat farms, these zoonotic food borne pathogen can transmitted from sheep husbandry to human via direct contact with uterine discharge and fetal membranes (Stanley, K. and Jones, K. 2003).

The current result revealed several species of bacteria were isolated from aborted placenta of women, the present study may indicated that the bacterial infection may considered one important cause of abortion in the women and the infection may reach the uterus from vagina, these evidence was agreement with result of Ahmed *et al.,* (2001). Who demonstrated that vaginal infections may associated with problem for mother and neonatal, in addition to normal opportunistic bacteria in the vagina may become virulent and induced BV.

The present result showed that most bacterial isolates from aborted placenta of women are similar to those isolated from aborted placenta of small ruminant, these result may indicated that infectious abortion in animal may considered a risk for human, these idea was agreement with observation of, Barkallah *et al.,* (2014). Who demonstrated that infectious abortion in ruminant do not only considered a problem in animal husbandry as a result of economic loss but cause infectious risk to humans and other animals

The present study showed that the *Salmonella* spp. form third bacterial isolates from aborted placenta of small ruminant may indicated these bacteria cause abortion in small ruminant, these idea was in consistent with, Hopper, (2015) who reported that Salmonella spp can cause sporadically abortion in the bovine.

Identification Information	Card:	GN			Lot Number:	2410655103			Expires:	Sep 12, 2019 13:00 CDT									
	Completed:	Feb 20, 2018 12:20 CST			Status:	Final			Analysis Time:	4.88 hours									
Organism Origin	VITEK 2																		
Selected Organism	99% Probability				Pseudomonas aeruginosa														
	Bionumber:	0043053003500240							Confidence:	Excellent identification									
SRF Organism																			
Analysis Organisms and Tests to Separate:																			
Analysis Messages:																			
Contraindicating Typical Biopattern(s)																			
Biochemical Details																			
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-		
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-		
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+		
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	-		
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-		
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-		
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-		
58	O129R	-	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-					

Fig. 4: Reveal *Pseudomonas aeruginosa* Identification by used VITEK®2.

The present result showed that the *P. aeruginosa* form high percentage of bacterial isolated from aborted placenta of women followed by *K. pneumonia* and *Staph. aerus,* these result may indicated that these pathogen associated with abortion in women, Filho. *et al.*, (2010). Reported that the main pathogens isolated from women bacterial vaginosis (BV), were *Pseudomonas* spp., *Staphylococcus aureus*, Coagulase-Negative *Staphylococci* (CoNS, *Acinetobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Proteus* spp., *Enterobacter* spp.,

Streptococcus agalactiae and *Escherichia coli* and they suggested that controlled these bacterial infection associated with reducing still birth, sterility and abortion.

Also these bacteria were isolated from BV in women by Razzak. *et al.*, (2011) in Iraq, (Marrazzo *et al*., 2008) in Seattle and (Larsen and Monif, 2001) in Omaha.

The current result showed that *P. aeruginosa* form the high bacterial isolates 15(75%) of aborted women placenta followed by *Klebsilla pneumonia* 6(30%) and *Staphylococcus aureus,* 4(20%), these result may indicated that *P. aeruginosa* associated with bacterial infection abortion in the women and these pathogens are common opportunistic bacteria in woman vagina particularly other opportunistic bacteria were isolated from aborted placenta of women in the present study, these observation was in consistent with result of Ranjit *et al*., (2018) who recorded that *Staph.* spp. form the high bacterial isolated from bacterial vaginosis followed by *Pseudomonas* spp. that form, 7.8% BV cases, followed by many other Gram negative bacteria, namely, *E. coli, Acinetobacter* spp., *Proteus* spp., *Klebsiella* spp., *N. gonorrhoeae, C. koseri a*nd *Enterobacter* spp.

However, *Pseudomonas* spp. is considered important opportunistic pathogen in the vagina which changes in their virulence factors associated with minor alteration in the vaginal environment and it considered primary pathogen in the urinary tract infection (Puri. *et al.*, 2006)

Also isolated of Staph aureus and *Streptococcus* spp. from aborted placenta of women was similar to result of other authors who isolated these bacteria from BV of women include (Al-Mousawi *et al.*, 2006) in Iraq (Maghsoudi *et al*., 2006) in Pakistan and (Tiyyagura *et al*., 2012) in India.

The present study showed that Gram positive cocci, *Enterococcus* spp. were isolated also, these result was agreement with Ranjit. *et al*., (2018) who isolated these bacteria from BV at 3.9%, also (Masood *et al.*, 2009) in Pakistan isolated these bacteria from BV, these bacteria may cause infection in impair immune system patients however, numerous bacterial isolated from aborted placenta in these study may indicated that inappropriate condition in the genital tract of the aborted women that lead to activated opportunistic bacteria and subsequently infected uterus of pregnant patient and abortion.

The biochemical tests, vitek®2 were used in this study to confirm all collected positive samples, because VITEK®2 Quick and precise methods have always been needed in the medical field to correctly identify the agent of infection. Automated systems for diagnosis of infectious pathogen such *P. aeruginosa* (Lucky *et al*., 2019).

The *P. aeroginosa* strain using in the present study expressed resistant to most antibiotic examination, these result may indicated these strain which isolated from aborted placenta of women and small ruminant characterized by multidrug resistant, these result may supported information of Peng *et al.,* (2014). Who found that the *P. aeruginosa*

Characterized by multiantibiotic resistance

Multi antibiotic resistant of *P. aeroginosa* to Ciprofloxacin, Levofloxacin, Tobramycin, Impenem, Cefapime Piperacillin + tazobactam were 50%, 30%, 40%, 40%, 60%, 60% respectively and these percentages agree with Yayan *et al.,* (2015) who revealed that resistance pattern were seen in (24.0%-70.4%, 24.5%-34.6%, 17.2-52.0%, 27.3%-55.6%, 50%) respectively and the percentage of Ciprofloxacin, Levofloxacin, Tobramycin, Impenem, Amikacin, aztreonam disagreement with Viren *et al.,* (2008) who revealed resistance pattern were (69.64%, 62.5%, 66.07%, 19.64%, 50%, 71.43%) respectively but; that approve with Viren *et al.,* (2008). Who showed resistance to Cefapime and ofloxacin (69.64%) for each one. While the results agreement with Philip *et al.,* (2009) who revealed resistance pattern was seen with ciprofloxacin, levofloxacin, Impenem (41-44%, 44%, 31%) but; Cefapime, aztreonam disagreement that showed disk diffusion results was 27-29%, 12-22% respectively. In addition disagreement with Tamil-Selvi and Sevanan, (2011) show 100% resistance to ciprofloxacin, levofloxacin and 66.6% for Impenem. but; Amikacin agree at 66.6%.

Haleem *et al.,* (2011) showed disk diffusion result for ciprofloxacin, Amikacin, Piperacillin+tazobactam was 31.26%, 39.5%, 20.08% respectively which were disagreement, except Polymyxin B was 60.41% was agree.

The percentage of Ciprofloxacin agreement with Marisa *et al.,* (2015) who recorded that 42.6%.

The susceptibility of *P. aeroginosa* to antibacterial agents include ciprofloxacin 26.79%, ofloxacin 26.79%, Tobramycin 32.14%, Cefapime 30.36% these agreement with Javiya *et al.,* (2008) but; disagreement with these antibiotic agent which the percentage was for levofloxacin 35.71%, Impenem 78.57%, Amikacin 48.21%. Ciprofloxacin agreement with Siva–Gowri *et al.,* (2009) at 26.79% but; disagreement with others antibacterial agents and disagreement with Yayan *et al.,* (2015) who revealed resistance pattern was seen with levofloxacin 39.6%, Tobramycin 44%, Cefapime 81%, Impenem 70%, Piperacillin+tazobactam 70% and polymyxin B 100% except Ciprofloxacin that agreement which was 29%.

References

Ahmed, S., T. Lutalo, M. Wawer, D. Serwadda, N.K. Sewankambo, F. Nalugoda and M. Kiddugavu (2001). HIV incidence and sexually transmitted disease prevalence associated with condom use: a population study in Rakai, Uganda. *Aids.*, **15(16):** 2171-2179.

Al-Zubaidi, S.F. (2015). Antibiotic susceptibility of bacteria isolated from the genital system of cows in Al-Hilla, Iraq.

Al-Mousawi, J.K.N., H.R. Tarish and M.M.K. Al-Saadi (2006). Microbiological study of Bacterial vaginosis among pregnant women in AL-Diwaniya city. *The Medical Journal of Basrah University,* **24(1&2):** 45-49.

Bajmocy, E., B. Fazekas and L. Fodor (1987). Mass outbreak of abortion associated with Campylobacter fetus in sheep flock. *Magyar Allatorvosok Lapia.*, **42(8):** 467-470.

Barkallah, M., Y. Gharbi, A.B. Hassena, A.B. Slima, Z. Mallek, M. Gautier and I. Fendri (2014). Survey of infectious etiologies of bovine abortion during mid-to late gestation in dairy herds. *PLoS One*, **9(3):** e91549.

Benie, C.K.D., G. Nathalie and D. Adjéhi (2017). Prevalence and Antibiotic Resistance of Pseudomonas aeruginosa Isolated from Bovine Meat, Fresh Fish and Smoked Fish. *Arch. Clin. Microbiol.,* **8(3)**.

Bizzarro, S., B.G. Loos, M.L. Laine, W. Crielaard and E. Zaura (2013). Subgingival microbiome in smokers and non smokers in periodontitis: an exploratory study using traditional targeted techniques and a next generation sequencing. *Journal of clinical periodontology.,* **40(5):** 483-492.

Driscoll, J.A., S.L. Brody and M.H. Kollef (2007). The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs.*, **67(3):** 351-368.

Filho, D.S.C., C.G. Diniz and V.L. Silva (2010). "Bacterial vaginosis: clinical, epidemiological and microbiological features," *HURevista Juiz de Fora.*, **36(3):** 223-230.

Giles, R.C., J.M. Donahue, C.B. Hong, P.A. Tuttle, M.B. Petrites-Murphy, K.B. Poonacha and T.W. Swerczek (1993). Causes of abortion, stillbirth and perinatal death in horses: 3,527 cases (1986-1991). *Journal of the American Veterinary Medical Association,* **203(8):** 1170-1175.

Gressler, L.T., A.P. Kowalski, C. Balzan, C. Tochetto, S.D.A. Botton, M.G. Ribeiro and A.C. De Vargas (2014). Co-infection by avirulent *Rhodococcus equi* and *Klebsiella oxytoca* as a cause of atypical abortion in a thoroughbred mare 4479-4487. *J.M.M. Case Reports.*, **1(3)**.

Hines, T.H. (2007). *Rhodococcus equi*. In Equine Infectious Diseases, 281-295. Edited by D.C. Sellon & M.T. Long. Missouri: Elsevier.

Holler, L.D. (2013). Diagnosis and control measures for opportunist infectious causes of reproductive failure. Proceedings of applied reproductive strategies in beef cattle,. Dealing with pregnancy and birth losses, 205-208.

Hopper, R.M. (2015). Fetal disease and abortion: diagnosis and causes. In: Bovine reproduction, Chapter 54, 1st edn. Wiley-Blackwell, Oxford, 481-517.

Javiya, V.A., S.B. Ghatak, K.R. Patel and J.A. Patel (2008). Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology.,* **40(5):** 230.

Lacasta, D., L.M. Ferrer, J.J. Ramos, J.M. González, A. Ortín and G.C. Fthenakis (2015). Vaccination schedules in small ruminant farms. *Veterinary microbiology.,* **181(1-2):** 34-46.

Larsen, B. and G.R. Monif (2001). Understanding the bacterial flora of the female genital tract. *Clinical Infectious Diseases.,* **32(4):** e69-e77.

Macpherson, M.L. (2005). Treatment strategies for mares with placentitis. *Theriogenology.,* **64(3):** 528-534.

Maghsoudi, R., A. Danesh, N. Kabiri, M. Setorki and M. Doudi (2014). Prevalence of the genital tract bacterial infections after vaginal reconstructive surgery. *Pakistan Journal of Biological Sciences.,* **17 (9):** 1058-1063.

Marisa, H., H. Didier, P. Cecile, C. Pascal, G. Christophe, M. Jean-Yves and B. Xavier (2015). Population structure and antimicrobial susceptibility of *Pseudomonas aeruginosa* from animal infections in France. *B.M.C. Vet. Res.,* **11:** 9.

Markey, B.K., F.C. Leonard, M. Archambault, A. Cullinane and D. Maguire (2014). Clinical Veterinary Microbiology. 2nd ed. Mosby Elsevier. 275-288.

Marrazzo, J.M., K.K. Thomas, T.L. Fiedler, K. Ringwood and D.N. Fredricks (2008). Relationship of specific vaginal bacteria and bacterial vaginosis treatment failure in women who have sex with women: a cohort study. *Annals of Internal Medicine.,* **149(1):** 20.

Masood, S.N., S.E.E.M.A. Mumtaz and M.U.S.H.A.R.A.F. Jahan (2009). Pattern of normal vaginal flora in healthy married non-pregnant women. *Pak. J. Surg.,* **25:** 128-131.

Moehario, L.H., H.P. Boestami, D. Edbert, E. Tjoa and T. Robertus (2019). Automation for the identification of *Pseudomonas aeruginosa*: Comparison of TDR-300B, VITEK® 2 and VITEK®-MS. BioRxiv, 510107.

Odumosu, B.T., O. Ajetunmobi, H. Dada-Adegbola and I. Odutayo (2016). Antibiotic susceptibility pattern and analysis of plasmid profiles of *Pseudomonas aeruginosa* from human, animal and plant sources. *Springer Plus.*, **5(1):** 1381.

Peng, Y., J. Bi, J. Shi, Y. Li, X. Ye, X.Chen and Z. Yao (2014). Multidrug-resistant Pseudomonas aeruginosa infections pose growing threat to health care–associated infection control in the hospitals of Southern China: A case-control surveillance study. *American journal of infection control.,* **42(12):** 1308-1311.

Pereira, S.G. and O. Cardoso (2014). Mobile genetic elements of Pseudomonas aeruginosa isolates from hydrotherapy facility and respiratory infections. *Clinical Microbiology and Infection.,* **20(3):** O203-O206.

Philip, D., J.L. Daniel, Wolter and D. Nancy (2009). Antibacterial-Resistant *Pseudomonas aeruginosa:* Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. *Journal List Clin. Microbiol. Rev.,* **22(4)**.

Puri, N., B. Jha, B. Lekhak and R.C. Adhikari (2006). Study on the incidence of urinary tract infection in diabetic patients and the prevalence of multidrug resistant strains among the bacterial pathogens isolates (Dissertation, thesis). Post Graduate Dissertation Submitted to the Central Department of Microbiology, Tribhuvan University, Kathmandu, India, 07-15.

Ranjit, E., B.R. Raghubanshi, S. Maskey and P. Parajuli (2018). Prevalence of bacterial vaginosis and its association with risk factors among non-pregnant women: a hospital based study. *International journal of microbiology*.

Razzak, M.S.A., A.H. Al-Charrakh and B.H. AL-Greitty (2011). Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginitis. *North American journal of medical sciences.*, **3(4):** 185.

Roest, H.I., J.J. Tilburg, P. Vellema, F.G. Van zijderveld, C.H. Klaassen and D. Raoult (2011). The Q fever epidemic in The Netherlands: history, onset, response and reflection. Epidemiology and Infection. **139:** 1-12.

Ruiz-Roldán, L., A. Bellés, J. Bueno, J.M. Azcona-Gutiérrez, B. Rojo-Bezares, C. Torres and C. Seral (2018). *Pseudomonas aeruginosa* isolates from Spanish children: occurrence in faecal samples, antimicrobial resistance, virulence and molecular typing. *Bio. Med. Research International*.

Sadikot, R.T., T.S. Blackwell, J.W. Christman and A.S. Prince (2005). Pathogen–host interactions in Pseudomonas aeruginosa pneumonia. *American journal of respiratory and critical care medicine*, **171(11):** 1209-1223.

Sargison, N.D., K. Dun, C.D. Penny, F. Howie and J.R. Thomson (2001). Ovine placentitis and abortion associated with a verotoxigenic strain of Escherichia coli.

Sela, S., O. Hammer-Muntz, O. Krifucks, R. Pinto, L. Weisblit and G. Leitner (2007). Phenotypic and genotypic characterization of *Pseudomonas aeruginosa* strains isolated from mastitis outbreaks in dairy herds. *Journal of Dairy Research*., **74(4):** 425-429.

Siva-Gowri, P., A. Samat and R. Mohamed (2009). Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital. *Journal List Malays. J. Med. Sciv.,* **16(2)**.

Stanley, K. and K. Jones (2003). Cattle and sheep farms as reservoirs of Campylobacter. *Journal of applied microbiology.,* **94:** 104-113.

Tiyyagura, S., M. Taranikanti, S. Ala and D.R. Mathur (2012). Bacterial vaginosis in Indian women in the reproductive age group. *International Journal of Biomedical Research.,* **3(8):** 371.

Tamil-Selvi, S. and M. Sevanan (2011). Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* from *Diabetes Patients with Foot Ulcers*., **1(4):** 95-99.

Vidal, S., K. Kegler, H. Posthaus, V. Perreten, S. Rodriguez-Campos (2017). Amplicon sequencing of bacterial microbiota in abortion material from cattle. US National Library of Medicine, *National Institutes of Health.,* **(48):** 64.

Viren, A., J.B. Somsuvra, R.G. Kamlesh and A. Jagruti (2008). Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, *India Journal List Indian J. Pharmacolv.,* **40(5)**.

West, D.M. (2002). Ovine abortion in New Zealand. *New Zealand veterinary journal.,* **50(3):** 93-95.

Yayan, J., B. Ghebremedhin and K. Rasche (2015). Antibiotic resistance of *Pseudomonas aeruginosa* in pneumonia at a single university hospital center in Germany over a 10-year period. *Plos one*, **10(10):** e0139836.