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 triturate 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

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

<table>
<thead>
<tr>
<th>Percentage % from total positive no. (32)</th>
<th>Positive isolation of bacterial species</th>
<th>Number of sample</th>
<th>Source of placenta sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>24%</td>
<td>12</td>
<td>50</td>
<td>Animal small ruminant (ewes and she goat)</td>
</tr>
<tr>
<td>40%</td>
<td>20</td>
<td>50</td>
<td>Women</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Percentage %</th>
<th>Positive no. for each Spp. from 20 positive isolate</th>
<th>Bacteria species</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>4</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Serratia fonticola</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>Salmonella spp.</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>Citrobacter freundii</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Granulicatella adiacens</td>
</tr>
<tr>
<td>15%</td>
<td>3</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Ochromobacter anthracpic</td>
</tr>
<tr>
<td>75%</td>
<td>15</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>30%</td>
<td>6</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Staphylococcus cohnii</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Citrobacter youngae</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Chromobacterium violaceum</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Achromobacter xylosoxidans</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Kocuria kristinae</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>Pantoena spp.</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>Enterococcus fecalis</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Staphylococcus xylosus</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>Streptococcus spp.</td>
</tr>
<tr>
<td>10%</td>
<td>2</td>
<td>E. coli</td>
</tr>
<tr>
<td>5%</td>
<td>1</td>
<td>Proteus vulgaris</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Percentage %</th>
<th>Positive no. for each Spp. from 20 positive isolate</th>
<th>Bacteria species</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>6</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>33.3%</td>
<td>4</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>33.3%</td>
<td>4</td>
<td>Citrobacter freundii</td>
</tr>
<tr>
<td>50%</td>
<td>6</td>
<td>Klebsiella pneumonia</td>
</tr>
<tr>
<td>33.3%</td>
<td>5</td>
<td>E. Coli</td>
</tr>
<tr>
<td>33.3%</td>
<td>4</td>
<td>Pantoena spp.</td>
</tr>
<tr>
<td>41.6%</td>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>33.3%</td>
<td>4</td>
<td>Salmonella spp.</td>
</tr>
<tr>
<td>16.6%</td>
<td>2</td>
<td>Achromobacter xylosoxidans</td>
</tr>
<tr>
<td>25%</td>
<td>3</td>
<td>Campylobacter jejuni</td>
</tr>
<tr>
<td>25%</td>
<td>3</td>
<td>Bacillus spp.</td>
</tr>
<tr>
<td>33.3%</td>
<td>4</td>
<td>Streptococcus spp.</td>
</tr>
</tbody>
</table>
Bacterial Isolation and Identification from Aborted Placenta of Women and Ewes and Goat in Al-Fallujah City

and non-lactose fermenter, on Blood agar, the colonies were large, irregular in shape, grey in color and surrounded by a zone of β-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.

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

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

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. aureus, 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 vaginosi (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 and 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. aeruginosa 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. aeruginosa to Ciprofloxacin, Levofloxacin, Tobramycin, Imopenem, 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, Imopenem, 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 Imopenem. 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. aeruginosa 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%, Imopenem 78.57%, Amikacin 48.21%. Ciprofloxacin agreement with Siva–Govri 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%, Imopenem 70%, Piperacillin+tazobactam 70% and polymyxin B 100% except Ciprofloxacin that agreement which was 29%.
References


