การปนเปื้อนเชื้อแบคทีเรียและการดื้อต่อยาปฏิชีวนะของเชื้อ *Escherichia coli* ที่แยกได้จากน้ำเชื้อพ่อสุกร

Bacterial Contamination and Antibiotic Resistance of *Escherichia coli* Isolated from Boar Semen

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บทคัดย่อ: การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อ จำแนกชนิดและปริมาณเชื้อแบคทีเรียที่ปนเปื้อนอยู่ในน้ำเชื้อพ่อสุกร และทดสอบความไวต่อยาปฏิชีวนะของเชื้อ *Escherichia coli* (E. coli) ที่แยกได้ โดยนำตัวอย่างน้ำเชื้อพ่อสุกรพันธุ์ดูร็อค จำนวน 10 ตัว มาแยกและหาปริมาณเชื้อด้วยเทคนิค Enriched method และ Direct method พบว่า มีการปนเปื้อนของเชื้อแบคทีเรียในทุกตัวอย่างน้ำเชื้อ (100 %, n=10) โดยพบทั้งแบคทีเรียแกรมบวกและแบคทีเรียแกรมลบ ซึ่งจากการจำแนกชนิดเชื้อจุลินทรีย์ด้วยวิธี Biochemical test พบชนิดของแบคทีเรียแกรมลบ ได้แก่ *Escherichia coli* (90%, n=9), *Edwardsiella tarda* (10%, n=1), *Klebsiella pneumonia* (20%, n=2), *Providencia stuartii* (10%, n=1), และ *Escherichia coli* (inactive) (20%, n=2) และพบแบคทีเรียแกรมบวก ได้แก่ *Staphylococcus* spp. (100%, n=10) และ *Streptococcus* spp. (10%, n=1) ทั้งยังพบว่า ปริมาณการปนเปื้อนของแบคทีเรียแกรมบวก (4.00×102 ถึง 8.50×103CFU/ ml) มีมากกว่าแบคทีเรียแกรมลบ (1.33×102 ถึง 4.17×103 CFU/ ml) ในทุกตัวอย่างของน้ำเชื้อพ่อสุกรและจากการทดสอบความไวต่อยาปฏิชีวนะด้วยวิธี Disk-diffusion method กับเชื้อ *Escherichia coli* จำนวน 15 สายพันธุ์ ที่แยกได้จากตัวอย่างน้ำเชื้อ ด้วยยาปฏิชีวนะจำนวน 6 ชนิด ได้แก่ Ampicillin, Ceftazidime, Cerfotaxime, Imipenem, Meropenem และ Colistin พบว่า เชื้อสามารถดื้อต่อยา Ampicillin ได้มากที่สุด (93.33%) รองลงมา คือ ยา Colistin (53.33%) แต่ในขณะเดียวกันยาชนิดอื่นๆ สามารถยับยั้งเชื้อได้ทั้งหมด

คำสำคัญ: การดื้อยาปฏิชีวนะ, การปนเปื้อนแบคทีเรีย, น้ำเชื้อพ่อสุกร

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ABSTRACT: The aims of this study were to isolate and identify the bacterial contamination and conduct antibiotic sensitivity of *Escherichia coli* (*E.coli*) isolated from boar semen. Bacterial contamination was investigated in 10 samples of boar semen by enriched and direct methods. The result showed that all semen samples (100%, n=10) were contaminated with both gram-negative and gram-positive bacteria. In fact, an identification using biochemical test indicated boar semen contaminated with gram-negative bacteria including *Escherichia coli* (90%, n=9), *Edwardsiella tarda* (10%, n=1), *Klebsiella pneumonia*e (20%, n=2), *Providencia stuartii* (10%, n=1, *Escherichia coli* (inactive) (20%, n=2), and gram-positive bacteria including *Staphylococcus* spp. (100%, n=10) and *Streptococcus* spp. 10% (n=1). The results revealed that the presence of gram-positive was higher than gram-negative bacteria in all samples (4.00×102 to 8.50×103 and 1.33×102 to 4.17×103 CFU/ ml, respectively). The 15 strains of E. coli were tested for antibiotic sensitivity with 6 antibiotics including Ampicillin, Ceftazidime, Cerfotaxime, Imipenem, Meropenem and Colistin using disk-diffusion method and found that most bacteria resisted to ampicillin (93.33%) and Colistin (53.33%). Meanwhile, there were no any strains (0%) resisted to the other antibiotics (Ceftazidime, Cerfotaxime, Imipenem nor Meropenem).
Keywords: Antibiotic resistance, Bacterial Contamination, Boar semen

Introduction

Microorganism contamination of boar semen had the deleterious influenced on sperm viability and litter size (Althouse and Lu, 2005; Martin et al., 2010). The sources of contamination were come from animal origin including fluid, hair, skin, respiratory system, and feces and environment such as water, feed, bedding material, un-sterilized equipment, and housing arrangement system (Bresciani et al., 2014). Moreover, both gram-negative and gram-positive bacteria have been isolated from boar semen, most frequently, which were *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pseumoniae), Pseudomonas aeruginosa (P.aeruginosa), Proteus vulgaris (P. vulgaris), Serratia marcescens (S. marcescens*), *Staphylococcus aureus (S. aureus)* and *Streptococcus pneumoniae (S. pneumoniae)* (Althouse et al., 2008; Martin et al., 2010). To solving of this problem, hygienic semen collection was respected and antimicrobial agents (antibiotics) were widely used in semen extenders for bacteria growth prevention and long-time preservation (Speck et al., 2014). However, bacteria resistance to antibiotics commonly used in boar semen extenders has been reported (Althouse and Lu, 2005). Then, the aims of the present study were to investigate the isolation and identification of bacterial contaminants and to determine the antibiotic susceptibility of *E.coli* isolated from boar semen.

Methodology

In this study, 10 samples of boar semen were collected from Duroc breed provided by a private farm. Gloved hand technique was used for collection of semen. All semen samples were analyzed for the presence of bacterial contamination using direct method by plating on the Mac Conkey Agar, Manitol Salt Agar and Luria-Bertani Agar (Martin et al., 2010; Kateete et al., 2010) . Bacteria were counted on agar plates by preparing the 5-fold serial dilution. After incubation at 37ºC overnight, the colony were calculated as colony forming units per milliliter (CFU/ ml). Bacterial isolates were

identified using standard microbiological procedures such as growth and colonial characteristics, gram staining, cellular morphology, and biochemical test (Kovacs, 1956; Lowrance et al., 1969; Sutter and Carter, 1972; Miller and Wright, 1982; Leclercq et al., 2001). Enterobacteriaceae were analyzed on website Identification of Enterobacteriaceae members according to P.N. Sridhar Rao (http://www.microrao.com/entero_ident.htm?fbclid=IwAR0IU_z9Q3lLwVQVU1F)

Antibiotic susceptibility tests were performed with the disk-diffusion method, evaluated on the basis of the criteria employed by the Clinical and Laboratory Standard Institute (CLSI) (CLSI, 2008). In this study 6 antibiotics were tested on 15 strains of E.coli isolated from boar semen. The list of antibiotics was Ampicillin (AMP), Ceftazidime (CTX), Cerfotaxime (CAZ), Imipenem (IPM), Meropenem (MEM) and Colistin (CT).

Results and discussion

The results showed that 10 samples of boar semen were contaminated with both gram-positive and gram-negative bacteria (Table 1). For gram-positive bacteria, *Staphylococcus* spp. was present in all semen samples (100%) whereas Streptococcus spp. was detected in 1 sample (10%). Of 10 samples, gram-negative bacteria, 9 were contaminated with *Escherichia coli* (90%), 1 with Edwardsiella tarda (10%), 2 with Klebsiella pneumonia (20%), 1 with Providencia stuartii (10%) and 2 with *Escherichia coli* (inactive) (20%). The number of gram-positive and gram-negative bacteria varied from 4.00×102 to 8.50×103 and 1.33×102 to 4.17×103 CFU/ ml, respectively. The antibiotic resistance data of isolated 15 strains of *Escherichia coli* was shown in Table 2. *Escherichia coli* isolates showed 93.33%

Table1 Microorganisms (CFU/mL) isolated from boar semen samples (n=10).

Sample	Gram negative bacteria		Gram positivebacteria	
	Species	Total bacteria/ sample (CFU/ ml)	Species	Total bacteria/ sample (CFU/ ml)
1	*Escherichia coli* *Klebsiella pneumoniae*	1.67×10^3	*Staphylococcus* spp.	3.83×10^3
2	*Escherichia coli* *Escherichia coli* (inactive) *Klebsiella pneumoniae*	1.67×10^2	*Staphylococcus* spp.	4.17×10^3
3	*Escherichia coli*	1.67×10^2	*Staphylococcus* spp.	4.00×10^2
4	Unknown	1.33×10^2	*Staphylococcus* spp.	4.67×10^3
5	*Escherichia coli*	2.33×10^2	*Staphylococcus* spp.	6.00×10^2
6	*Escherichia coli* *Escherichia coli (inactive)*	1.00×10^3	*Staphylococcus* spp.	4.50×10^3
7	*Escherichia coli*	1.00×10^3	*Staphylococcus* spp.	8.50×10^3
8	*Escherichia coli*	1.17×10^3	*Staphylococcus* spp.	1.00×10^3
9	*Escherichia coli* *Edwardsiella tarda*	3.83×10^3	*Streptococcus* spp. *Staphylococcus* spp.	5.67×10^3
10	*Escherichia coli* *Providencia stuartii*	4.17×10^3	*Staphylococcus* spp.	3.67×10^3

(n=14) resistance to AMP and 53.33% (n=8) to CT.

In this study, the bacterial contamination was found 100% of semen samples and different bacterial species were isolated including *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Edwardsiella tarda*, *K. pneumonia*, *P. stuartii* and *E. coli* (inactive). These were the common genera found in boar semen (Althouse and Lu., 2005; Bresciani et al., 2014; Gaczarzewicz et al., 2016; Martin et al., 2010). Bacterial contamination of boar semen, especially *E. coli,* has been associated with deleterious effects on semen quality and litter size at birth (Althouse et al., 2008; Martin et al., 2010).

Our study, 15 strains of *E. coli* were isolated and conducted the antimicrobial susceptibility testing. We found that mostly *E. coli* resisted to AMP and partly to CT. Likewise, Bresciani et al. (2014) reported that E. coli isolated from boar semen resisted to AMP (75%) and CT (95%), in Italy. The emergence of multiple resistance gram negative bacteria to colistin and other

Table 2 Antibiotics resistance of *Escherichia coli* isolated from boar semen.

Antimicrobial Susceptibility Testing (n=15)			
Antibiotics	Susceptible	Intermediate	Resistant
AMP (10 µg)	1 (6.67%)	0 (0%)	14 (93.33%)
CTX (30 µg)	15 (100%)	0 (0%)	0 (0%)
CAZ (30 µg)	15 (100%)	0 (0%)	0 (0%)
IPM (10 µg)	15 (100%)	0 (0%)	0 (0%)
MEM (10 µg)	15 (100%)	0 (0%)	0 (0%)
CT (10 µg)	7 (46.33%)	0 (0%)	8 (53.66%)

Conclusion

In the present study can conclude that the gram-negative and gram-positive bacteria were contaminated in different degree of boar semen. Besides, the isolated 15 strains of *Escherichia coli* resisted to ampicillin and colistin.

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