

Microbial count and prevalent bacteria isolated from semen of four breeds of boars extended with moringa leaf extracts in beltsville thawing solution



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Abstract

Antimicrobials are added to extenders to control growth of microbes contaminating semen. However, non-therapeutic use of antibiotics may contribute to antibiotic resistance. Moringa oleifera leaf has been identified as a natural antibiotic source hence, this study was designed to compare the efficacy of Moringa Leaf Extract (MLE) as alternative to penicillin and streptomycin. Forty ejaculates each from four boar Large White, Landrace, Duroc and Gene Converter 750 breeds of pigs were collected using gloved hand technique and extended with MLE incorporated at 0, 0.25, 0.50, 0.75 and 1.00g/250mL of BTS with 0g having penicillin (0.28g) and streptomycin (0.28g). Semen samples were analysed for microbiological contamination, bacterial count and isolate identity was deduced from the Bergey's manual of Systematic Bacteriology. 0gMLE inclusion had lower (0.75cfu/100µL) bacterial count ($p<0.05$) compared to 0.25, 0.50 and 0.75gMLE with 55.75, 52.75 and 41.00cfu/100µL respectively. Large white exhibited lowest bacterial count (21.80cfu/100µL). Interactions between MLE inclusions and breed revealed absence of bacteria ($P<0.05$) in 0gMLE (0.00 cfu/100µL) for all breeds except Gene Converter 750 (3.00cfu/100µL) where *Alcaligenes* spp was found. 1.00gMLE showed bacterial lowering effect although not as much as synthetic antibiotics. Gram -ve bacteria were isolated in all ejaculates with MLE (0.25-1.00g). *Pseudomonas* spp, *Aeromonas* spp, *Enterococcus* spp, *Alcaligenes* spp, *Serratia marcescens*, *Escheria coli* were isolated in all breeds. *Acinetobacter baumannii*, *Buchholdera mallei*, *Citrobacter* spp and *Pseudomonas fluorescens* were also isolated. Prospects of MLE as a natural antibiotic source that could be useful in swine semen processing was established due to the dose dependent bacteria load lowering response gotten in the MLE groups. However, since the inclusion group with synthetic antibiotics had better bacteria lowering capacity than 1.00gMLE, we inferred that MLE concentration for swine semen processing may need to be increased in further studies.

Key words: Moringa, ejaculate, antimicrobial, antibiotic, extenders and bacterial contamination.

Nombre de micro-organismes et bactéries prédominantes isolées du sperme de quatre races de verrats, dilué avec des extraits de feuilles de moringa dans la solution de décongélation de Beltsville



Résumé

Des agents antimicrobiens sont ajoutés aux diluants afin de contrôler la prolifération des micro-organismes contaminant le sperme. Cependant, l'utilisation non thérapeutique d'antibiotiques peut contribuer à la résistance aux antibiotiques. La feuille de Moringa oleifera ayant été identifiée comme une source naturelle d'antibiotiques, cette étude a été conçue pour comparer l'efficacité de l'extrait de feuilles de moringa (MLE) en tant qu'alternative à la pénicilline et à la streptomycine. Quarante éjaculations provenant respectivement de quatre verrats des races Large White, Landrace, Duroc et Gene Converter 750 ont été prélevées à l'aide de la technique de la main gantée, puis diluées avec du MLE incorporé à des concentrations de 0, 0,25, 0,50, 0,75 et 1,00 g/250 ml de BTS, le groupe témoin (0 g) contenant de la pénicilline (0,28 g) et de la streptomycine (0,28 g). Les échantillons de sperme ont été analysés pour détecter toute contamination microbiologique ; le nombre de bactéries a été déterminé et l'identité des isolats a été établie à l'aide du manuel de bactériologie systématique de Bergey. L'inclusion de 0 g de MLE a donné lieu à un nombre de bactéries plus faible (0,75 ufc/100 µL)

($p < 0,05$) par rapport aux doses de 0,25, 0,50 et 0,75 g de MLE, qui présentaient respectivement 55,75, 52,75 et 41,00 ufc/100 μ L. La race « Large White » présentait la charge bactérienne la plus faible (21,80 cfu/100 μ L). Les interactions entre les teneurs en MLE et les races ont révélé l'absence de bactéries ($P < 0,05$) dans la teneur de 0 g de MLE (0,00 ufc/100 μ L) pour toutes les races, à l'exception de la race « Gene Converter 750 » (3,00 ufc/100 μ L), chez laquelle des bactéries du genre *Alcaligenes* spp. ont été détectées. La teneur de 1,00 g de MLE a montré un effet réducteur sur la charge bactérienne, bien que moins important que celui des antibiotiques de synthèse. Des bactéries Gram-négatives ont été isolées dans tous les éjaculats traités au MLE (0,25-1,00 g). Des espèces de *Pseudomonas*, *Aeromonas*, *Enterococcus*, *Alcaligenes*, *Serratia marcescens* et *Escherichia coli* ont été isolées chez toutes les races. Des espèces d'*Acinetobacter baumannii*, *Buchholdera mallei*, *Citrobacter* et *Pseudomonas fluorescens* ont également été isolées. Les perspectives du MLE en tant que source naturelle d'antibiotiques pouvant s'avérer utile dans le traitement du sperme porcin ont été établies grâce à la réduction de la charge bactérienne, proportionnelle à la dose, observée dans les groupes traités au MLE. Cependant, étant donné que le groupe de contrôle ayant reçu des antibiotiques de synthèse présentait une meilleure capacité de réduction bactérienne que le groupe traité à 1,00 g de MLE, nous en avons déduit que la concentration en MLE pour le traitement du sperme porcin devrait peut-être être augmentée dans le cadre d'études ultérieures.

Mots-clés : Moringa, éjaculat, antimicrobien, antibiotique, diluants et contamination bactérienne.

Introduction

Bacteriospermia, the presence of bacteria in semen remains a challenge in processing boar semen; it leads to decreased shelf life, reduced motility, sperm agglutination as well as reproductive problems in inseminated females (Althouse *et al.*, 2000). The boar itself is a source of bacterial contamination in the ejaculate, likely due to the preputial diverticulum (Althouse and Evans, 1994). However, appropriate hygiene during semen collection can decrease contamination (Althouse *et al.*, 2005). Sources of contamination could also include human handlers, water used for semen extension, collection and laboratory equipment (Althouse and Lu, 2005). To determine the source of contamination, raw and corresponding extended ejaculates should be cultured. Samples from water used in semen processing and swab of laboratory equipment should also be cultured to determine the source of potential bacterial threat. Even if there are no known bacterial problem on site, semen should be cultured regularly to monitor for contamination (Reicks, 2003). In order to reduce the contamination, most boar semen extender contains at least one antibiotic with the aim of inhibiting the growth of bacteria; however,

there are disadvantages to this practise both for sperm quality and for the environment. The antimicrobials may have detrimental effect on sperm survival, thus limiting the choice of agents that can be added to semen extenders. In a bid to reduce the toxicity caused by bacteria, various highly potent broad spectrum antibacterial agents are used. However, use of antibiotics can contribute to the development of antibiotic resistance and this can in turn be transferred to other bacteria in other host species (Vickram *et al.*, 2017) one of such extender with the inclusion of broad spectrum antimicrobials is the Beltsville Thawing Solution (BTS). The BTS was initially developed for frozen semen and later adapted to condition fresh swine semen at 15-18°C. Due to challenges of antibiotics resistance, unavailability, expensive price and the ban on some antibiotics, the use of natural plant sources to boost and preserve semen is on the increase. *Moringa oleifera* as a plant has an impressive range of medicinal uses with high nutritional value throughout the world. *Moringa* contains pterygospermin which has powerful antibacterial effects (Rao *et al.*, 1946; Fahey *et al.*, 2005). The leaves of *Moringa oleifera* have been reported to be a valuable source of macro and micronutrients, rich source

of β -carotene, protein, vitamin C, calcium, potassium and act as a good source of natural antioxidants (Siddhuraju and Becker, 2003). A number of medicinal properties attributed to different parts of *Moringa* have been recognized by both Ayurvedic and Unani systems of medicines (Mughal *et al.*, 1999). Components of *Moringa* preparations have been reported to have antibacterial activity which includes 4-(4'-O-acetyl- α -L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin and 4-(α -L-rhamnopyranosyloxy) benzyl glucosinolate (Fahey *et al.*, 2005). The benefit for the treatment or prevention of disease or infection that may accrue from either dietary or topical administration of *Moringa* preparations (e.g. extracts, decoctions, poultices, creams, oils, emollient, powders) are not quite well known (Palada, 1996) hence, culturing semen extended in BTS with MLE replacing antibiotics and determining the bacterial count, identifying prevalent bacteria isolated in semen of four exotic breeds of boar is encouraging. Identifying the effect of varying breeds on microbial load and prevalence will add to repositories of knowledge in swine artificial insemination (AI) and provide insights on whether breed influences bacterial count and prevalence. This result will thereby provide basis for breed selection for AI. Ngo *et al.* (2025) identified that breeds were among factors that may influence the diversity and richness of boar seminal microbiome. Notwithstanding, the authors underscored that research on seminal microbiome remains relatively limited. Fisher *et al.* (2026) reported insignificant influence of breed, age or season on raw-semen total bacterial count when 92 boars of different breeds were evaluated. Fisher *et al.* (2026) noted that the condition of boar stud and collection protocols appeared more relevant in lowering bacterial contamination than breed. It would be vague to draw conclusion on the influence of breed on bacterial count and prevalence since Ngo *et al.* (2025) and Fisher *et al.* (2026) reiterated that breed may influence microbial-community composition through host-genetic and

physiological effects, but total bacterial load appears to be dominated by individual-boar, management, hygiene and environmental factors.

Materials and methods

Experimental site

The experiment was carried out at the Artificial Insemination Centre of the Livestock Improvement Programme of the Institute of Agricultural Research and Training, Ibadan (IAR & T) in Ikenne, Ogun state, West Africa (Latitude 6°55' N, longitude 30°35' E).

Experimental animals and management

The study was approved by the Institute of Agricultural Research and Training (IAR&T), Animal Welfare and Ethics Committee of Obafemi Awolowo University. A total of 16 exotic boars of different breeds and of similar age (2year) and weight (150-200kg) namely: 4 Large White, 4 Landrace, 4 Duroc and 4 Gene Converter 750 (GC 750) were used for the experiment. The boars were housed intensively and fed balanced ration according to NRC (2012) maintenance requirement for breeding boars.

Methanolic extraction of *Moringa oleifera* leaf

Fresh *Moringa oleifera* leaves were obtained from Bora farm of the Institute of Agricultural Research and Training (I.A.R & T) Ibadan. The leaves were air dried over a two weeks period and 2000 g of the powdered leaves was extracted with 500 mL of 70% methanol for 72 hours. The mixture was filtered and extracted using a soxhlet apparatus which was set at 60°C (Afolabi *et al.*, 2013).

Preparation of the BTS extender

The Beltsville thawing solution (BTS) was constituted from: Glucose 39.1g, Sodium bicarbonate 1.32g, Tri-sodium citrate 1.32g, EDTA 1.32g, Potassium chloride 0.79g, Streptomycin 1.1g, Penicillin (IU/LI) 1.1g and 1000 mL distilled water (Omodewu *et al.*, 2021). The *Moringa* leaf extracts were included at 0g MLE (BTS+antibiotics), BTS+0.25gMLE, BTS+0.50gMLE, BTS+0.75gMLE and BTS+1.00gMLE.

Semen Collection

Semen was collected from boars weekly using gloved-hand technique as described by King and Macpherson (1973). Forty ejaculates were collected from each breed between 8.00 and 10.00am over a 10 weeks. The semen was extended with the constituted Beltsville thawing solution at a ratio 1:10. The extended semen were transferred into labeled tubes with the varying inclusion levels of MLE and then transferred in a climate box maintained at 18°C to the laboratory and evaluated immediately.

Bacteriological Evaluation of Extended Semen

All semen samples were analysed for the presence of microbiological contamination by plating on the following media: Nutrient Agar, MacConkey agar (Oxoid), Blood agar, Sabouraud agar (Oxoid, UK), Manittol Salt Agar and nitrate reduction medium. The media were prepared according to the manufacturer's instructions and the plates were incubated in aerobic conditions at 37°C.

Microbial count

The microbial count of the extended semen with varying levels of Moringa leaf extract was examined as described by Cheesebrough (2006). The phenotypic properties of isolated bacteria were investigated using different methods, depending on type; the identification process was based on the use of the Gram staining and Analytical Profile Index systems (API 10S, API Staph and API 20NE; bioMe'rieux, France). Motility test, Catalase test, Oxidase test, Indole test, Starch hydrolysis, Methyl red test, Voges-Proskauer test, Citrate utilization test, Nitrate reduction test and Sugar Fermentation were all carried out as described (Garrity, 2007).

Biochemical characterization of isolates

Pure cultures of bacteria isolates were subjected to various biochemical tests to determine the probable identity of the bacteria isolates. The result of each test was recorded and the probable identity of the isolates was deduced with reference to the Bergeys Manual of Systematic Bacteriology (2007). Bacteriological characteristics were determined as described by Bezuidenhout *et al.* (2019).

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) in a 5 × 4 factorial design using SAS (2003). Mean values that were significant ($P < 0.05$) were separated using Duncan's Multiple Range Test (DMRT).

Statistical model

$$Y_{ijkl} = \mu + A_i + B_j + A*B_{ij} + E_{ijkl}$$

Y_{ijkl} : Dependent variable

μ : Population mean

A_i : Moringa extract effect ($i = 0, 0.25, 0.50, 0.75, 1$)

B_j : Breed effect ($j = \text{Large white, Landrace, Duroc and GC 750}$)

$A*B_{ij}$: Interaction between A_i and B_j

E_{ijkl} : Residual Error

Results and Discussion

The effect of the inclusion of varying levels of Moringa leaf extract (MLE) on bacterial count of exotic boar semen extended with BTS

According to Table 1, the effect of including Moringa leaf extract (MLE) as replacement for synthetic antibiotic in BTS on bacterial count of boar semen was significant ($p < 0.001$), 0.25g MLE (55.75cfu/100µL), 0.50g MLE (52.75cfu/100µL) and 0.75g MLE (41.00cfu/100µL) had comparable values which were significantly higher than 0g MLE (0.75cfu/100µL) and 1g MLE (18.25cfu/100µL). This clearly suggests that the antibacterial effect of MLE is in a dose dependent manner as 1.00g MLE had significantly lower bacterial count compared to the lower inclusion rates, this is in line with the reports of Prabu *et al.* (2019) which noted the dose dependent nature of MLE. This results also highlights the efficacy of synthetic antibiotics over MLE at 1.00g indicating the need to further increase the MLE dosage to combat the negative effects of bacteria. The need to optimize MLE concentration is clearly obvious as authors such as Hammad *et al.* (2019) and Amao and Sokunbi (2021) have reported positive results in semen characteristics of Friesian bull during different preservation stages and semen quality indices of Red Sokoto bucks when the synthetic

antibiotics in the semen diluents were replaced with Moringa leaf extract, respectively.

Table 1: The effect of the inclusion of varying levels of Moringa leaf extract (MLE) on bacterial count of exotic boar semen extended with BTS

Treatment (g)	0g MLE	0.25g MLE	0.50g MLE	0.75g MLE	1.00g MLE	±SEM	P-Value
Bacteria count (cfu/100µL)	0.75 ^c	55.75 ^a	52.75 ^a	41.00 ^a	18.25 ^b	4.261	0.0001

Means with different superscript ^{a, b} and ^c (±SEM) in the same row are significantly different (p<0.001)

The effect of breed on bacterial count of semen extended with Beltsville thawing solution (BTS) with varying levels of MLE

The effect of breed on bacterial count of semen extended with Moringa leaf extract (MLE) in Beltsville thawing solution (BTS) is presented in Table 2. The bacterial count in Large White (21.80cfu/100µL) was significantly lower (p<0.01) compared to Landrace (43.60cfu/100µL) and Duroc (38.40cfu/100µL) while GC 750 was intermediate (31.00cfu/100µL). This result suggests breed-specific responses to the treatment which have been highlighted by researchers such as Marques *et al.* (2017) and Hensel *et al.* (2023). The Large White boars semen exhibiting the lowest bacterial count suggests that their semen would be less prone to bacterial contamination when treated with MLE in BTS compared to other breeds. This could imply a superior quality of semen in terms of microbial load, which is crucial for maintaining semen integrity and fertilizing potential during storage and artificial insemination (Althouse and Lu, 2005). The higher bacterial counts observed in Landrace and Duroc indicates an increased bacterial concentration compared to Large White which could indicate a greater susceptibility to bacterial contamination or less efficacy of the MLE in BTS in controlling bacterial growth in these breeds. Authors such as Althouse *et al.* (2000) clearly reports that elevated bacteria count in boar semen would potentially impact the longevity and fertility of the semen, suggesting strict hygiene protocols. For breeds such as the Landrace and Duroc it is clearly imperative that a much more higher

concentration of MLE or using other alternative additives for these breeds would be required. The Gene converter (GC750) (31.00cfu/100µL) had bacterial count that was similar to the other breeds. The bacterial count in GC750 breed (31.00 cfu/100µL) suggests a moderate level of bacterial contamination, indicating that the efficacy of the treatment with MLE in BTS vary across breeds. The noticed breed-associated differences indicates that boar genetics could contribute to variations in the microbial burden of their semen, potentially because of varying reproductive-tract microbial ecology, epithelial defence, seminal-plasma indices and susceptibility to contamination. Breed has been identified as a potential determinant of diversity and richness of the boar seminal microbiome; however, bacterial contamination can be complex and multi-dimensional influenced by hygiene, housing, semen collection, processing and storage conditions (Ngo *et al.*, 2025). The elevated bacterial load observed in Landrace and Duroc semen may be of practical relevance to semen quality due to bacterial proliferation that negatively affect sperm function through sperm agglutination, membrane disruption, acrosomal damage, oxidative stress and reduced motility, thereby potentially shortening the useful storage period of extended semen (Lopez Rodriguez *et al.*, 2017)). Martin *et al.* (2010) observed an association between bacteriospermia, especially spermagglutinating *Escherichia coli*, and decreased litter size, highlighting the reproductive importance of microbiological semen quality. However, the present breed effect should be interpreted conservatively due to a recent study that reveals

that total bacterial counts may vary considerably between individual boars and may not necessarily differ significantly among breeds. Thus, the higher counts recorded in Landrace and Duroc in the present study may

reflect a combination of breed-related susceptibility and experimental/environmental factors rather than an inherent breed characteristic (Fischer *et al.*, 2026).

Table 2: The effect of breed on the bacterial count of semen extended with Beltsville thawing solution with varying levels of MLE

Breed	Landrace	Large white	Duroc	GC 750	±SEM	P-Value
Bacteria count (cfu/100µL)	43.60 ^a	21.80 ^b	38.40 ^a	31.00 ^{ab}	4.260	0.0127

Means with different superscript ^a and ^b (±SEM) in the same row are significantly different (p<0.05)

The interactive effect between breeds and varying inclusion levels of Moringa leaf extract on bacterial count of semen extended with Beltsville thawing solution.

Table 3 shows the interactive effect between breeds and varying inclusion levels of Moringa leaf extract on bacterial count of semen extended with Beltsville thawing solution. The results highlights the differential responses of each breed to the varying levels of MLE, suggesting that both breed and MLE concentration play significant roles in influencing bacterial counts. The values obtained significantly differed (p<0.05).

Duroc semen had bacterial count that was highest (p<0.05) at 0.25g (73.00 cfu/100µL) and 0.50g MLE (61.00 cfu/100µL) indicating that the lower MLE concentrations were less effective in reducing bacterial contamination. Significant reduction in bacterial count was observed at 0.75g MLE (39.00 cfu/100µL) and further at 1.00g MLE (19.00 cfu/100µL), indicating a dose dependent response in the semen of the Duroc pig treated with MLE which is in synergy with the report of Hammad *et al.* (2019). At 0g MLE, no bacterial growth was recorded, suggesting that the inclusion of synthetic antibiotics (penicillin and streptomycin) had sufficient antimicrobial effect to prevent bacterial growth, this validates the submission of Vickram *et al.* (2017) that synthetic antibiotics are added to semen with the aim of inhibiting the growth of bacteria.

In Large White boar semen, bacterial count increased at 0.25g MLE (24.00 cfu/100µL) and remained high at 0.50g and 0.75g MLE (36.00 cfu/100µL). A significant reduction (p<0.05) was seen at 1.00g MLE (13.00 cfu/100µL), similar to the initial 0g MLE condition with no bacterial growth. This indicates that higher concentrations of MLE (1.00g) are more effective for Large White semen samples which are in line with the reports of Amao and Sokunbi (2021) that highlights the potential of MLE in semen preservation.

The trend noticed in Landrace boar semen were similar to those of the Large White, high bacterial counts was noticed at 0.25g MLE (83.00 cfu/100µL), followed by 0.50g (57.00 cfu/100µL) and 0.75g MLE (62.00 cfu/100µL). A significant reduction (p<0.05) was seen at 1.00g MLE (16.00 cfu/100µL), indicating the need for higher MLE concentrations for effective bacterial control in this breed. Similar to other breeds, 0g MLE had no bacterial growth.

In Gene Converter 750 (GC750) semen, bacterial count was lowest (p<0.05) at 0g MLE (3.00 cfu/100µL) resulting from the inclusion of synthetic antibiotics (Vickram *et al.*, 2017) and increased significantly at 0.25g (43.00 cfu/100µL) and 0.50g MLE (57.00 cfu/100µL). Counts were reduced at 0.75g (27.00 cfu/100µL) and 1.00g MLE (25.00 cfu/100µL) also validating the reports of Amao and Sokunbi (2021) of the antibacterial potential of

MLE, this results also suggests breed specific responses to the effect of MLE in BTS.

Table 3: The interactive effect between breeds and varying inclusion levels of Moringa leaf extract on bacterial count of semen extended with Beltsville thawing solution.

Breed	0g MLE	0.25g MLE	0.50g MLE	0.75g MLE	1.00g MLE	±SEM	P-Value
Duroc (cfu/100µL)	0.00 ^c	73.00 ^a	61.00 ^a	39.00 ^b	19.00 ^{bc}	9.12	0.013
Large White (cfu/100µL)	0.00 ^b	24.00 ^{ab}	36.00 ^a	36.00 ^a	13.00 ^{ab}	5.31	0.013
Landrace (cfu/100µL)	0.00 ^b	83.00 ^a	57.00 ^a	62.00 ^a	16.00 ^b	10.76	0.013
GC 750 (cfu/100µL)	3.00 ^b	43.00 ^{ab}	57.00 ^a	27.00 ^{ab}	25.00 ^{ab}	7.63	0.013

Means with different superscript ^a, ^b and ^c (±SEM) in the same row are significantly different (p<0.05)

Identification of bacteria isolates

The results of bacteria isolation obtained in this study showed that 0g MLE which had synthetic antibiotics inhibited the growth of bacteria in all boar semen except the Gene Converter 750, which had *Alcaligenes spp.* Inhibition of bacteria growth observed in 0g MLE attests to the potency of synthetic antibiotics as reported by Vyt *et al.* (2007). The presence of gram –ve bacteria *Alcaligenes spp* in Gene Converter (GC) 750 could be breed dependent as reported by Kommisrud *et al.* (2002). Duroc semen with varying inclusions of MLE had *Alcaligenes spp* and *Acinetobacter iwoffii* at 0.25g, *Serratia marcescens* and *Alcaligenes spp* at 0.50g, *Citrobacter spp* and *E coli* at 0.75g inclusion while 1.00g MLE had *Pseudomonas auregenosa* and *Enterococcus spp.*

The semen samples of Large White had *Aeromonas spp* at 0.25, 0.50 and 0.75g inclusion of MLE while *Serratia marcescens* and *E coli* were isolated at 1.00g MLE. The Landrace boar semen had no growth at 0g MLE, while 0.25g MLE had *Buckholdera mallei*, 0.50g MLE had *Shewanella putrifaciens* and *Pseudomonas fluorescens*, 0.75g MLE had *Shewanella spp* and *Shewanella putrifaciens* while 1.00g MLE had *Pseudomonas Flourescens* and *Escherichia coli*. GC 750 at 0g MLE had *Alcaligenes spp*, 0.25g MLE and 0.50g MLE had *Acinetobacter baumanii* while *Alcaligenes spp* was isolated from 0.75g MLE. In 1.00g MLE, *Alcaligenes spp* and *Enterococcus spp* were the most prevalent.

Enterococcus spp, *Pseudomonas flourescens*, *Serratia marcescens*, *Citrobacter spp* were similar to bacteria reported by Althouse *et al.* (2000). Althouse *et al.* (2000) reported that potential sources of contamination in boar semen include Laboratory equipment, disposable and non-disposable supplies, powdered/reconstituted extenders, boars itself and the boar stud environment. *Alcaligenes spp*, *Acinetobacter iwoffii*, *Aeromonas spp*, *Buckholdera mallei*, *Shewanella putrifaciens*, *Acinetobacter baumanii* and *Alcaligenes spp* were different from bacteria noticed in the study by Althouse *et al.* (2000) and this could be as a result of ecological differences. The results from this study are in line with the research of Althouse and Lu (2005), Okazaki *et al.* (2010) and Kuster and Althouse (2016) that largely confirms the general view that gram-negative bacteria, especially from the *Enterobacteriaceae* family are most prevalent in boar semen. These results are consistent with those of other authors, compiled by Martin *et al.* (2010), who reported the 25 common bacteria genera identified in boar semen, the species *Pseudomonas*, *Staphylococcus*, *Proteus*, and *E. coli* were identified to occur most frequently. The isolates in these results suggest the need to optimize the MLE inclusions.

Table 4: The prevalent bacteria isolated from semen samples extended with varying inclusions of MLE in BTS

Breeds	MLE	Prevalent Isolates from sample with MLE
Duroc	T1 (0g)	No growth
	T2 (0.25g)	<i>Alcaligenes</i> spp, <i>Acinetobacter iwoffii</i>
	T3 (0.50g)	<i>Serratia marcescens</i> , <i>Alcaligenes</i> spp
	T4 (0.75g)	<i>Citrobacter</i> spp, <i>E. coli</i>
	T5 (1.00g)	<i>Pseudomonas auregenosa</i> , <i>Enterococcus</i> spp
LargeWhite	T1 (0g)	No growth
	T2 (0.25g)	<i>Aeromonas</i> spp
	T3 (0.50g)	<i>Aeromonas</i> spp
	T4 (0.75g)	<i>Aeromonas</i> spp
	T5 (1.00g)	<i>Serratia marcescens</i> , <i>E. coli</i>
Landrace	T1 (0g)	No growth
	T2 (0.25g)	<i>Buckholderia mallei</i>
	T3 (0.50g)	<i>Shewanella putrifaciens</i> , <i>Pseudomonas flourescens</i>
	T4 (0.75g)	<i>Shewnell</i> spp, <i>Shawanella putrifaciens</i>
	T5 (1.00g)	<i>Pseudomonas Flourescens</i> , <i>Escherichia coli</i>
GC 750	T1 (0g)	<i>Alcaligenes</i> spp
	T2 (0.25g)	<i>Acinetobacter baumanii</i>
	T3 (0.50g)	<i>Acinetobacter baumanii</i>
	T4 (0.75g)	<i>Alcaligenes</i> spp
	T5 (1.00g)	<i>Alcaligenes</i> spp, <i>Enterococcus</i> spp

*All the bacteria isolated are gram –ve , GC 750: Gene Converter 750

Conclusion

Penicillin and streptomycin were more effective against bacteria when compared to MLE. Semen processing protocols might need to be tailored towards breed to optimize bacterial control and semen quality. Large White boars exhibited better responses to MLE in BTS, making it a preferable choice for breeding programs while alternative or additional antibacterial measures might be necessary for Landrace and Duroc breeds. MLE showed antibacterial activity at 1.00 g/250 mL but did not match conventional antibiotics.

Recommendation

It is recommended that investigations be carried out on the prospects of utilizing MLE with a higher dosage to determine if a higher concentration could further reduce or inhibit bacteria population in boar semen.

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