ejpmr, 2022, 9(8), 01-11.

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ISOLATION AND IDENTIFICATION OF GRAM POSITIVE BACTERIA FROM PET RABBIT FAECES

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Article Received on 23/05/2022 **Article Revised on 13/06/2022** **Article Accepted on 03/07/2022**

ABSTRACT

Rabbit faeces consist of numerous species of bacteria comprising both gram positive and gram negative. These bacteria are actually present in the caecum microbiota that forms a complex ecosystem which plays an important role in maintaining the reliability of the rabbit's digestive health. The present study was conducted to examine the occurrence of gram positive bacteria in rabbit faeces. From 35 isolates, 14 strains of gram positive bacteria were isolated on the basis of Gram Staining technique from the rabbit faeces and distinctly labeled from RFS-01 to RFS-14. Afterwards these strains were identified as *Staphylococcus aureus, Lactobacilli* and *Enterococcus spp.* The percentages in incidence were 35.71% for both *Staphylococcus aureus* and *Lactobacilli*. While the *Enterococcus sp*. percentage in present study was 28.57%. By applying biochemical test and after culturing bacteria on selective agar the bacteria species were identified. The *Staphylococci* species named were culture on Phenol Red Mannitol Salt Agar supplemented with 7.5% NaCl. *Lactobacilli* isolated on MRS and Pfizer Selective *Enterococcus* Agar was confirmed the presence of *Enterococcus.* The biochemical tests that were performed in present study were Catalase test, Oxidase test and the Coagulase test. The results may contribute to the study about the presence of gram positive bacteria in the faeces of herbivorous animals as well as evaluate the chances of microbial transmission from pet rabbits.

KEYWORDS: Caecum microbiota, Gram staining, *Lactobacilli*, *Staphylococcus*, Catalase, Gram positive bacteria.

INTRODUCTION

Rabbits are found nearly every country in the world thatpositioned third in number to dogs and cats as companion animals. Rabbits are monogastric andextensively used for research models and conservation. Microbes can rise on the skin coat as well as in the GI and respiratory tracts. Role and composition of these bacterial colonies have been extremelydeliberated in past few years. The most colonized organ is the GI tract that has affluent nutrients which can be used by microbes.[1] Most food conversionproceedingsin rabbits happen in the caecum, which is thicklyinhabited with bacterias. The colonization and growth of microorganisms become fast under favorable conditions of gut.[2;3] The GI tract of rabbit is modified to course withhuge amounts of fiberloaded feed. Microbial fermentation also takes place in the caecum to guarantee nutrient supply.[4] As compared to other herbivores, the rabbit does not contain the enzymes crucial for complete digestion of the plant substrates. Early investigations of the GI tract of rabbit demonstrated that microbial community performs various remarkable tasks.[6]

Microorganisms in mammal's intestine and faeces have been studied for decades. A great amount of microbes (10^{13} to 10^{14} bacteria/ g) live in GI tract and faeces of mammals. The variety and density in rabbits is an abundant microbiota (10^{10} to 10^{12} bacteria/ g) in hard and soft faeces.[7;8;9] Thediscovery and explorationof this microbiota is a very tempting challenge. The constancy of caecum microbial fermentation is vital for rabbit health. Nutrients which go through the caecum facilitate colonization for abundant microbial population. The responsibility of thismicrobiota in the digestion is manifested by sacharrification of plant cell walls via bacterial enzymes, which is not probable by host animal digestive enzymes.[10;11] In addition, the GI tractof microflora provides certain nutrients that are helpful to the host. Though, a complete understanding of the stability and composition of the GI microfloraremains unfinished.[12]

Digestive disorders are the majorreason of mortality in commercial rabbits. Enteritis is the prime GI disorder cause diarrhea as a result of an imbalance microflora in the gut.[13] Previous studies exposed that the GImicrobiota of rabbits consists predominantly of

strictly anaerobic, Gram-negative bacteria belonging to the genus Bacteroides, while anaerobic sporulated Gram-positive bacteria are present in significantly lower numbers.[14] Lactic acid bacteria belong to the clostridial subdivision of the Gram-positive and are fermentative, strictly anaerobic or microaerophilic microorganisms that produce high amounts of organic acids as a consequence of their metabolism. Enterococci belong to the group of lactic acid bacteria; they are regular inhabitants of the intestine and serve as indicators of faecal contamination. Besides, probiotic properties have been claimed for certain enterococci.[16]

Facultative anaerobic bacteria isolated from the intestinal tract of rabbits belong to the Gram-positive genera *Bacillus*, *Enterococcus* and *Staphylococcus*. *S. aureus*is a facultative anaerobe so can grow under both aerobic and anaerobic conditions.[17;18] Staphylococci are gram-positive, catalase-positive, oxidase negative, facultatively anaerobic and non-motile cocci. The coagulase-positive *Staphylococcus aureus* are of particular importance as primary causes of specific diseases such as *S. aureus*cause mastitis in ruminants, equine botryomycosis; *S. intermedius*cause canine pyoderma.[19] Most coagulase positive Staphylococci are widespread bacteria pathogenic to both humans and animals and cause a number of severe infectious diseases.[19; 20] In a population-based survey performed in the United States, the prevalence of colonization with *S. aureus*was 31.6%. *S. aureus*can cause various non-food related health issues such as skin inflammations, mastitis, respiratory infections, wound sepsis and toxic shock syndrome.[21] Staphylococcal food poisoning symptoms generally have a rapid onset, appearing around 3 hours after ingestion (range 1–6 hours). Common symptoms include nausea, vomiting, abdominal cramps and diarrhea.[22]

Enterococci are Gram-positive, facultative anaerobic, catalase-negative lactic acid bacteria (LAB). *Enterococcus faecalis*is a Gram-positive, facultative anaerobic coccus that can survive under harsh conditions, including high salt concentrations and temperatures > 45°C. It is a member of the mammalian GImicrobiota but multidrug-resistant strains have been considered relevant causes of hospital-acquired and community related infections. The occurrence of enterococci in rabbit meat could be mostly originated from the environment.[23;24;25] Enterococcal surface protein (ESP) was highly associated with infection-derived isolates of *E. faecium*and *E. faecalis*. The prevalence of *E. faecalis*in sub-gingival samples of periodontitis patients ranges from as little as 1% to almost 50%.[26; 27]

Lactobacilli are widely distributed in nature. The presence of lactobacilli in fecal or intestinal samples has been traditionally demonstrated by bacteriological culture. Some species are believed to be members of the commensal flora of the intestinal tract of humans and animals.[28] Some *Lactobacillus* species have also received considerable attention with respect to their putative healthful properties when ingested as probiotics.[29] *Clostridium botulinum* is a ubiquitous Gram-positive, spore forming obligatory anaerobic bacterium that primarily inhabits soil, dust and organic matter such as feces of animals and man, slaughterhouse wastes, biogas plant residues, and bio-compost.[30] The present work concentrates on the incidence and propensity profiles for Gram positive bacteria species in rabbit faeces.

MATERIALS AND METHODS

PBS solution and media preparation

PBS was prepared by dissolving 8.0g NaCl, 0.2g KCl, 1.44g Na_2HPO_4 and0.24g KH_2PO_4 in one liter distilled water. The pH range was 7.1-7.3. After mixing salts it was poured (9mL) in test tubes. It was sterilized by autoclaving at 121°C for 15 minutes at 15lb/inch^2. After sterilization it was cooled at room temperature for further use.[31]

Nutrient agar powder (HIMEDIA) was prepared by dissolving 2.8g in 100 mL distilled water. Its pH was adjusted 7.4±2 with help of pH meter. It was sterilized by steam heat under pressure (121°C for 15 minutes at 15lb/inch^2). After cooling it was poured in sterile glass Petri plates within six inches of flame. The medium was allowed in the plates to solidify.[32]

Sampling and Isolation of bacteria

A total of 10 faecalsamples were carefully collected from ten healthy rabbits of both sexes and various ages. The study was conducted in the laboratory of the Department of Microbiology in the University of Veterinary and Animal Sciences, Lahore.

For the isolation of the bacteria, nutrient agar as a medium was poured into culture plates. Faecal material (1g) of rabbit was dissolved in 9ml of sterile PBS. The PBS containing test tubes were already sterilized by autoclaving. Serial dilutions (10 fold) were made from it. Pour a 100 µL suspension on nutrient agar plates with help of micropipette. It was uniformly spread on plate with help of an L-shaped sterile glass spreader. These agar plates were incubated at 37ºC for 24 hours.

Purification of colonies

After post incubation the plates were taken off from the incubator. The plate having discrete colonies was selected for purification. For purification single isolated colony was picked with platinum loop and it was streaked on sterile nutrient agar plate by using quadrant streak plate method. Here nutrient agar was still used for purification. These plates were incubated at 37ºC in the incubator for 24 hours. Similarly all colonies with different morphology were purified.[33]

Morphological Characterization

i. Colony Morphology

Quadrant streak plate method was used to get isolated colonies for the study of colony morphology. Colony shape, size, margins and colour of colony were observed with naked eye.[34]

ii. Microscopic Analysis

Microscopic analysis was done for further characterization of bacteria. Two types of staining procedures were applied that was gram staining and spore staining.

- **Gram Staining**

A thin bacterial smear was equipped on glass slide and fixed it by heat. The smear on a glass slide was covered with few drops of Crystal Violet for 1 minute. The slide was washed by distilled water. The smear was treated with few drop of Gram's Iodine and allowed to act for a minute. The slide was again washed in water and then decolorized in 95% ethyl alcohol for 30 seconds. After the smear is decolorized, it was washed with water without any delay. The smear was finally treated with few drops of counterstain such as safranins for one minute. The slide was washed in water; excess water was removed using a blotting paper, dried in air observing under microscope. Those bacteria that hold on to primary dye-iodine complex and remain violet are called Gram positive and those which get decolorized and subsequently take up counterstain (pink/red) are called Gram negative.[35]

Only those microbes were selected for further study which showed purple (violet staining reactor) in Grams staining.

- **Spore Staining**

Smears of bacterial culture (5 to 7 days incubated) were made on clean glass-slides, air-dried and heat-fixed. Smears were flooded with 0.75% aqueous malachite green solution and heated on the steam of water bath for 10 minutes. While heating on boiling water, care was taken that stain should not be dried out. After staining slides were washed with distilled water and counter-stained with 1-2 drops of safranin for 30 seconds. Bacterial smear was washed again, air dried and observed under oil immersion lens.[36]

Biochemical Characterization

- **Catalase Test**

Two circles were marked on the glass slides, one for test and other for negative control. H_2O_2 bottle was taken and place a drop of H_2O_2 on the slide. From sterile sticks, one stick was selected. One of the plates was chosen and then picked an isolated colony using the stick. The stick (with bacteria) was moved to the drop of H_2O_2 on the slide.The tip dragged into the drop of H_2O_2 on the slide and gently mixed into the solution. Observed for bubbling and the result was noticed either "positive" or "negative". Immediate bubble formation indicates production of O_2 and a positive result. The test was then repeated for the second plate and glass slide.[37]

- **Oxidase Test**

Filter paper disk was soaked with oxidase reagent. Transfer a loop full of pure bacteria to the disk by using sterilized loop.The disk was observed for up to three minutes. If the area of inoculation was turned in dark-blue or purple then the result was positive. If a color change was not occurring within three minutes, the result was negative.[38]

- **Coagulase Test**

It was performed by slide coagulation method. Dense suspensions of Staphylococci from culture were made on two ends of clean glass slide. One was labeled as "test" and the other as "control". The control suspension served to rule out false positivity due to auto agglutination. The test suspension was treated with a drop of citrated plasma and mixed well. Agglutination or clumping of cocci within 5-10 seconds was taken as positive. Some strains of *S.aureus*was might not produce bound coagulase, and such strains must be identified by tube coagulase test.[39]

- **Mackonkey Salt Agar**

Dehydrated medium of MSA (HIMEDIA) 51.153 grams was suspended in 100 ml distilled water. It was mixed thoroughly and boiled for 1 minute. After that it was sterilized by autoclaving at 15 lbs pressure (121°C) for 25 minutes. Then medium was cooled to 45-50°C and poured into sterile Petri plates. Then plates were incubated at pH 7.1+/-0.02 at 25˚C for 24 hours.

Confirmation on Selective Agar

The confirmation of bacterial specie was done by using selective agar.

i. Mannitol Salt Agar

Mannitol salt Agar was used as a selective agar for *Staphylococcus aureus.* Mannitol salt agar (BDH Anala R) was prepared by dissolving 6.66g in 100 mL distilled water. Its pH was attuned 7.4 ± 0.2 at 25°C with aid of pH meter. It was sterilized at 121°C for 15 minutes at 15lb/inch^2 by steam heat under pressure. After cooling within six inches of flame it was poured in sterilize glass Petri plates. The medium was allowed to harden in the plates.[40] The yellow colour colonies were observed after incubation.

ii. MRS Agar

MRS agar was used to confirm *Lactobacilli*. MRS (HIMEDIA) was prepared by dissolving 67.15 grams in 1000mL distilled water. With assist of pH meter the pH was adjusted 6.5±0.2 at 25°C. It was sterilized at 121°C for 15 minutes at 15lb/inch^2 by steam heat under pressure. After cooling it was surrounded by six inches of burn and poured in sterilize glass Petri plates. The medium was allowed to harden in the plates.[41] On MRS agar, there was off white colour colonies of bacteria were present.

iii. Pfizer Selective *Enterococcus* Agar

Similarly Pfizer nutrient agar was used for *Enterococci.* PSE Agar is used for selective isolation and cultivation of *Enterococci*. PSE Agar (HIMEDIA) was prepared by dissolving 57.75 grams in 1000 ml distilled water (Table: 3.13). With assist of pH meter the pH was adjusted 7.1±0.2 at 25°C. It was sterilized at 121°C for 15 minutes at 15lb/inch^2 by steam heat under pressure. After cooling it was poured in sterile glass Petri plates within six inches of flame. The medium was allowed to solidify in the plates.[42] The resulted colonies were blackening around the sides.

RESULTS

Isolation and Purification

Gram positive bacteria were isolated from rabbit faeces. Out of 10 samples 35 isolates were obtained by dilution method. The isolated bacteria were identified with morphology, microscopy and biochemical purification.

Selection of Gram Positive Bacteria

Gram staining was done for further identification. The bacteria which show purple colour of Gram stains were Gram positive and pink stained were gram negative. Out of 35 isolates, 14 were gram positive. These were selected for further analysis. The isolates strains were distinctly designated as RFS-01, RFS-02, RFS-03, RFS-04 and so on till RFS-14. The percentage of gram positive bacterial isolate is shown in Table 3.2.

Identification of bacteria

Identification of bacteria was done by applying biochemical test. The biochemical test that we were performed here were Catalase test, Oxidase test and the Coagulase test. The results for respective species were mentioned in the Table 3.2.

A total number of 14 species of gram positive bacteria were isolated from rabbit faeces. For further identification biochemical tests were applied. The species that give positive Catalase test were RFS-02, RFS-05, RFS-08, RFS-11 and RFS-13. While the species that show negative result for Catalase test were RFS-01, RFS-03, RFS-04, RFS-06, RFS-07, RFS-09, RFS-10, RFS-12, and RFS-14.All isolates reaction towards oxidation test was negative. The isolates RFS-02, RFS-05, RFS-08, RFS-11 and RFS-13 which were positive for Catalase test also show positive result for coagulase test. The coagulase test was not applicable on remaining isolates thar were RFS-01, RFS-03, RFS-04, RFS-06, RFS-07, RFS-09, RFS-10, RFS-12, and RFS-14.

Here we tentatively identified that the species RFS-02, RFS-05, RFS-08, RFS-11 and RFS-13 were *Staphylococcusaureus*, as they were catalase (positive), oxidase (negative) and coagulase (positive).

The remaining species RFS-01, RFS-03, RFS-04, RFS-06, RFS-07, RFS-09, RFS-10, RFS-12, and RFS-14 were other gram positive bacteria. For their further identification they were culture on selective agar media. As coagulase test was not applicable on these species so they may be *Enterococci, Lactobacilli* or someone else.

Confirmation on Selective Medium

Further confirmations were done by culture on selective agar.

Here for further identification the bacterial species were culture on selective agar. The prepared medium was red coloured and gel forms in Petri plates. The species named RFS-02, RFS-05, RFS-08, RFS-11 and RFS-13 were culture on MSA. This medium was resulted as Mannitol ferment. *Staphylococci* have the unique ability of growing on a high salt containing media. Isolation of coagulase-positive *staphylococci* on Phenol Red Mannitol Agar supplemented with 7.5% NaCl as already mentioned.

Two further selective medium was prepared for remaining bacteria culture. Firstly MRS agar was prepared, which medium was dark amber coloured. The remaining bacteria were culture on MRS Agar plates. *Lactobacilli* MRS medium was based on the formulation of deMan, Rogosa and Sharpe with slight modification. It was supported luxuriant growth of all *Lactobacilli*. *Lactobacilli* were microaerophillic and generally require layer plates for aerobic cultivation on solid media. *Lactobacilli* isolated on MRS Agar confirmed. These isolates were RFS-01, RFS-04, RFS-06, RFS-07 and RFS-14.

Pfizer Selective *Enterococcus* Agar was used for selective isolation and cultivation of *Enterococci.* This was light amber coloured with a bluish tinge forms in Petri plates. The importance of esculin hydrolysis in differentiating *Enterococci* and *streptococci* was first reported as streptococci do not exhibit esculin hydrolysis. Esculetin reacts with ferric ammonium citrate to form a dark brown to black coloured complex. Pfizer Selective *Enterococcus* Agar was confirmed the presence of *Enterococcus.* These isolates were RFS-03, RFS-09, RFS-10 and RFS-12.

From rabbit faecal samples 35 bacteria were isolated. The total number of gram positive species that were isolated was 14 in present study. By applying biochemical test and after culturing bacteria on selective agar the bacteria species were identified. The *Staphylococcus aureus* were 5(total isolate=14) in numbers. *Staphylococcus aureus* were comprised 35.71% in incidence in total positive bacteria isolates. The second specie was *Lactobacilli* that were 5(total isolate=14) in numbers. So, the lactobacilli were comprised a percentage of 35.71%. The *Enterococcus spp* were 4(total isolate=14) in numbers. And their specie percentage in present study was 28.57%.

According to chi-square analysis ($p>0.05$), species prevalence in rabbit faeces is non-significant (Table 3.5).

Table no: 3.1 Percentage of gram positive bacteria in Rabbit faeces.

Sample no	Total isolates	Gram Positive	Percentage
1	4	1	25%
2	3	1	33.34%
3	5	2	40%
4	3	1	33.34%
5	4	1	25%
6	3	2	66.67%
7	2	1	50%
8	3	0	0%
9	5	3	60%
10	3	2	66.67%
Total	**35**	**14**	**40%**

Table no: 3.2 Biochemical profiles of Gram positive Bacteria.

Isolates	Spore Staining	Catalase test	Oxidase test	Coagulase test	Growth on MaCConkey's agar
RFS-01	NA	-	-	-	+
RFS-02	NA	+	-	+	NA
RFS-03	-	-	-	NA	NA
RFS-04	NA	-	-	-	+
RFS-05	NA	+	-	+	NA
RFS-06	NA	-	-	-	+
RFS-07	NA	-	-	-	+
RFS-08	NA	+	-	+	NA
RFS-09	-	-	-	NA	NA
RFS-10	-	-	-	NA	NA
RFS-11	NA	+	-	+	NA
RFS-12	-	-	-	NA	NA
RFS-13	NA	+	-	+	NA
RFS-14	NA	-	-	-	+

(NA=Not Applicable)

Table 3.3 Results of growth on Selective agar.

Isolates	Selective Agar	Result	Identification
RFS-02 RFS-05 RFS-08 RFS-11 RFS-13	MSA Agar	Yellow Colour Mannitolfermentor	*Staphylococcus aureus*
RFS-03 RFS-09 RFS-10 RFS-12	MRS Agar	Off white Colonies Tolerate microaerophilic	*Lactobacilli*
RFS-01 RFS-04 RFS-06 RFS-07 RFS-14	Pfizer Selective *Enterococcus* Agar	Blackening around the colony Positive reaction, good luxuriant	*Enterococcus spp.*

Table 3.4 Percentage Prevalence of Bacterial species.

Isolates	Specie Name	Specie Number	Percentage
RFS-02 RFS-05 RFS-08 RFS-11 RFS-13	*Staphylococcus aureus*	5	35.71%
RFS-03 RFS-09 RFS-10 RFS-12	*Lactobacilli*	5	35.71%
RFS-01 RFS-04 RFS-06 RFS-07 RFS-14	*Enterococcus faecalis*	4	28.57%
Total Isolates = 14			100%

Table: 3.5 Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.000[a]	2	.223
Likelihood Ratio	3.819	2	.148
Linear-by-Linear Association	.000	1	1.000
N of Valid Cases	3		

6 cells (100.0%) have expected count less than 5. The minimum expected count is .33.

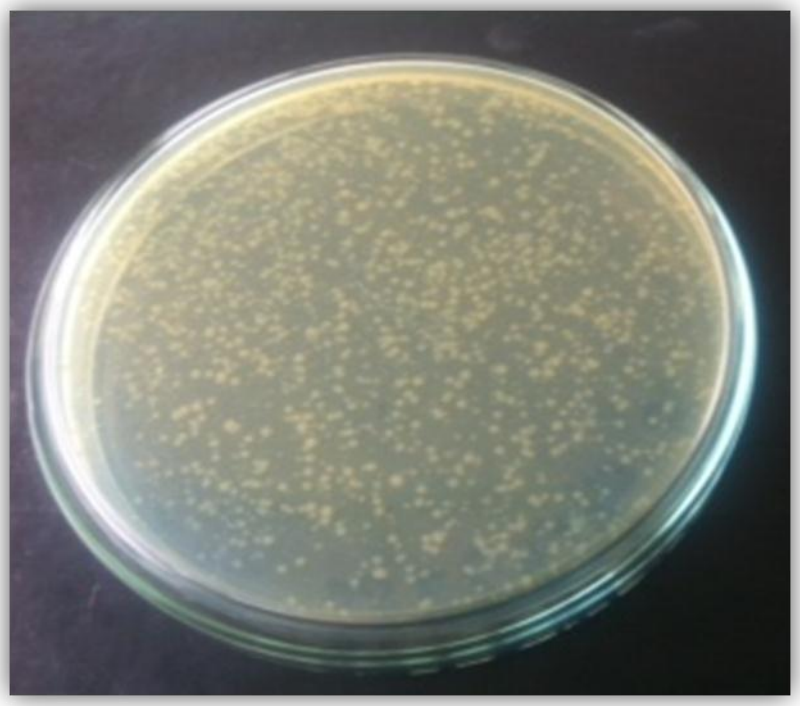

Figure 3.1: Primary Culture of faecal sample on Nutrient agar (10^5 dilutions)

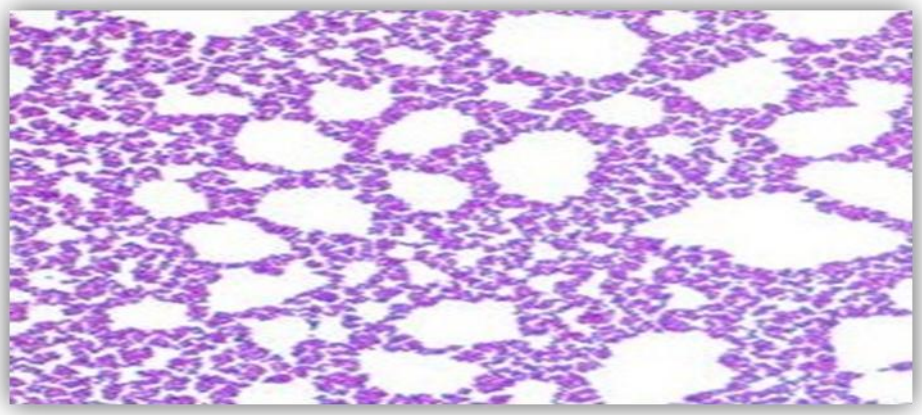

Figure 3.2: Microscopic view of a pure culture.

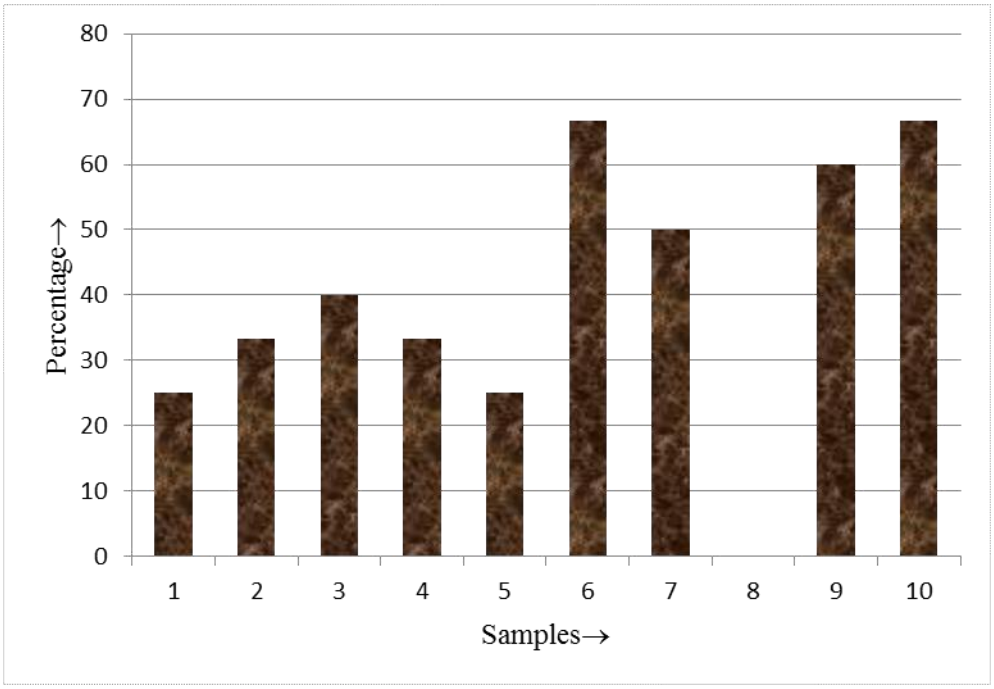

Figure 3.3: Graphical representation of Percentage of Gram positive Bacteria in Rabbit faeces.

Figure 3.4: Catalase test results positive.

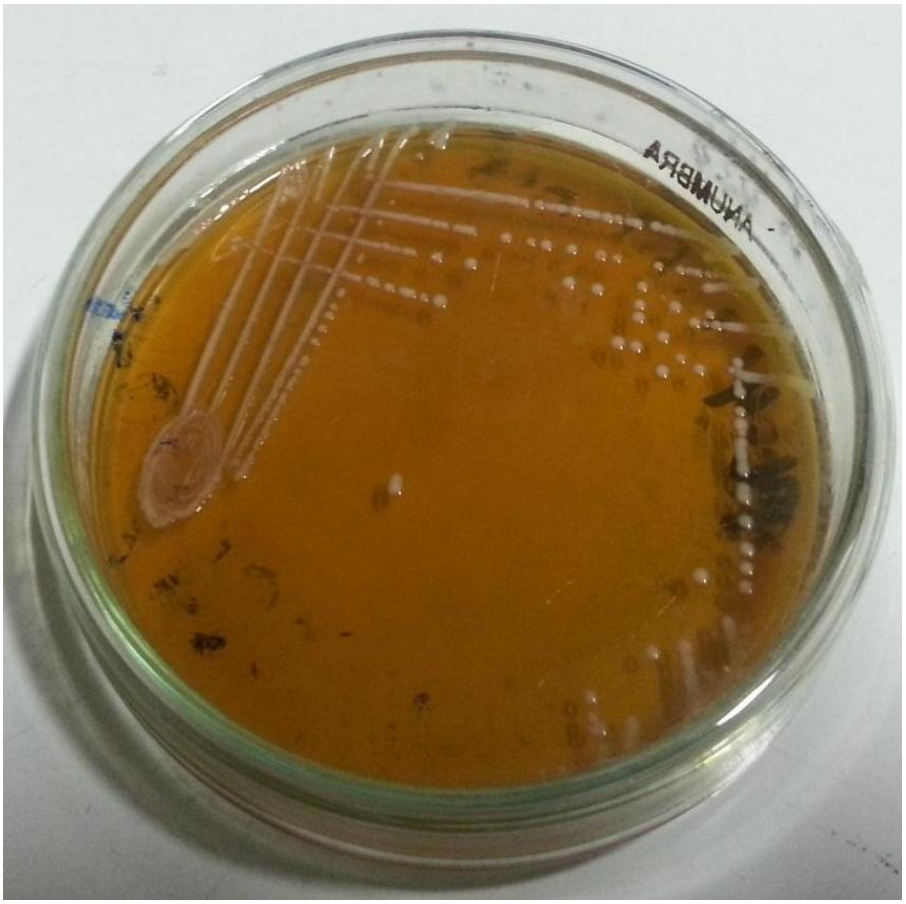

Figure 3.5: Lactobacilli (MRS Agar)

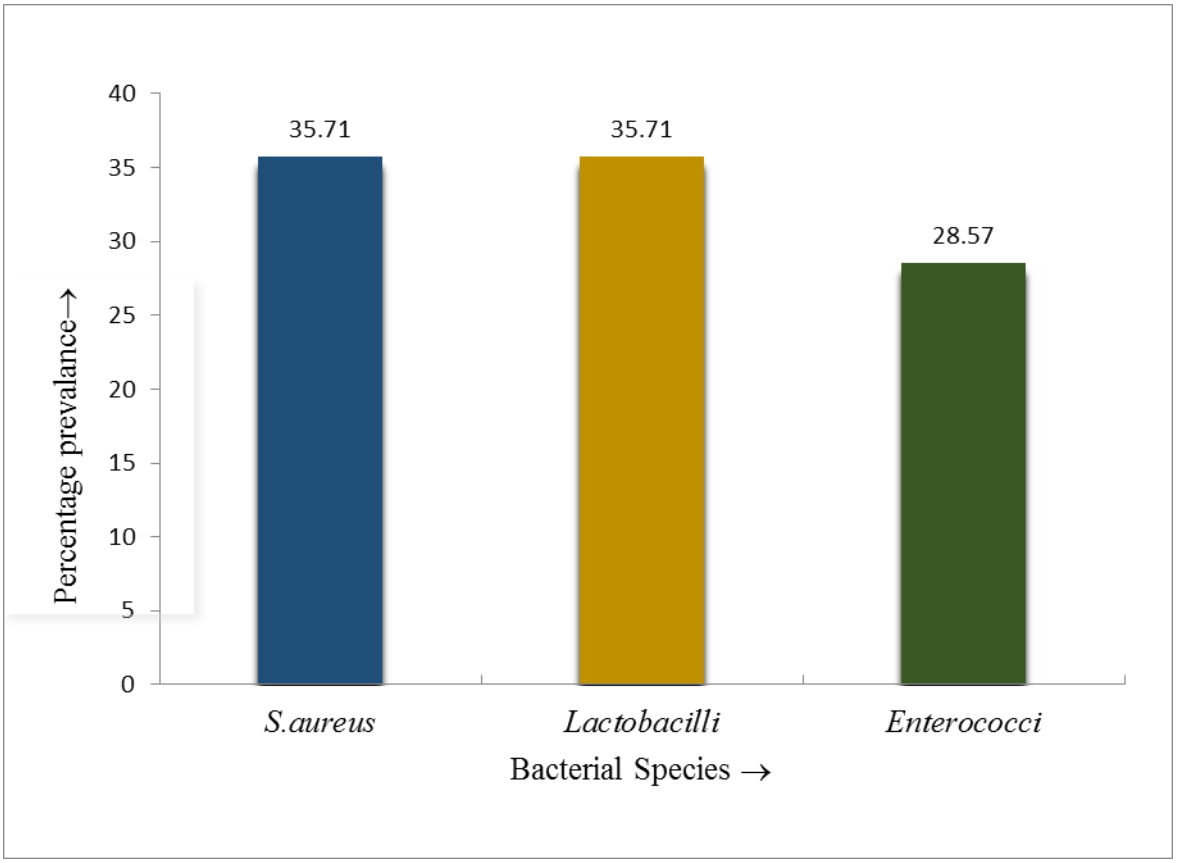

Figure 3.6: Graphical representation Percentage Prevalence of Bacterial specie.

DISCUSSION

In present study 10 samples of rabbit faeces were used from which total 35 bacteria were isolated. On the basis of Gram Staining 14 strains of gram positive bacteria were isolated from rabbit faeces and were identified as *Staphylococcus aureus, Lactobacilli and Enterococcus faecalis.* On the basis of gram staining 14 strains of bacteria were distinctly labeled from RFS-01 to RFS-14. The biochemical tests that were performed in present study were Catalase test, Oxidase test and the Coagulase test. The biochemical characteristics of these bacteria and further identification for confirmations of respective bacteria on selective medium were shown in Table3.2 and 3.3.

Lactobacilli isolated on MRS Agar and was confirmed their presence on Pfizer Selective Enterococcus Agar. The lactobacilli were comprised a percentage of 35.71% and their specie percentage in present study was 28.57%. A study related to this[43] worked onmicroflora of rabbits showed the presence of *Lactobacilli* and*Enterococci* were isolated from faeces. In present study *Staphylococcus aureus*were identified on the basis of biochemical characterization. For the identification of Staphylococcus*aureus*, catalase test (positive), oxidase test (negative) and coagulase test (positive) were used. They were culture on selective agar MSA forming yellow colour colonies. Their percentage in the faeces of rabbits that was demonstrated from a total number of 14 gram positive isolates was 35.71%. The finding of present study was revealed that *Staphylococcus aureus*were present in rabbit faeces. Another study[44] proved the occurrence of *Staphylococcus aureus* in rabbit faeces and its susceptibility to antibiotics. So it was revealed that both studies were closely related.

In present study biochemical tests apply on *Lactobacilli* were Catalase test (negative) and Oxidase test (negative). Lactobacilli cultured on MRS Agar. These isolates were named as RFS-01, RFS-04, RFS-06, RFS-07 and RFS-14. The specie *Lactobacilli* were 5(total isolate=14) in numbers. So, the *Lactobacilli* were comprised a percentage of 35.71%. The finding of present study was closely related to a previous study[45] that describe the *Lactobacilli* were present in the faecal samples and their number was decreased as rabbit become aged.

A study[46] deliberate to characterize the facultative anaerobic intestinal microbiota of healthy rabbits, especially enterococci, for the selection of potential probiotic strains. In present study as a selective agar Pfizer Selective Enterococcus Agar was used for isolation and cultivation of *Enterococci*. The presence of *Enterococcus faecalis* was confirmed by Pfizer Selective Enterococcus Agar. The isolates that named as RFS-03, RFS-09, RFS-10 and RFS-12 in present work were identified as *Enterococci.*The specie percentage in present study was 28.57%. Their biochemical characterization was done by using Catalase test (negative) and oxidase test (negative). The Coagulase test was not applicable on these bacteria. A study[47] revealed that the intestinal microbiota of rabbits consists predominantly of Gram-negative bacteria, while anaerobic sporulated Gram-positive bacteria are present in significantly lower numbers. The similar findings were recorded in the present study also.

CONCLUSION

The caecal microbial community has been mainly studied using culture techniques, to demonstrate gram positive bacterial species occurrence. From 35 isolates 14 strains of gram positive bacteria were isolated from the rabbit faeces (samples=10) and were identified as *Staphylococcus spp*(total counts=5), *Lactobacilli* (total counts=5), *Enterococcus spp*(total counts=4). This study may helpful to determine antimicrobial susceptibility profiles of respective isolates (*Staphylococcus spp* and *Enterococcus spp*) either the isolates were sensitive to antibiotics or not. The *Lactobacilli* were isolated from rabbit faeces can be evaluated for probiotic properties.

Abbreviations: Gastrointestinal (GI), lactic acid bacteria (LAB), PBS (Phosphate-buffered saline), MSA (Mannitol Salt Agar) and PSE (Pfizer Selective *Enterococcus).*

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