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Diversity of foodborne bacteria isolated from different sources of minced meat samples in Riyadh, Saudi Arabia

MAJED ALBOKARI[1*], MEHMET MUTLU[2], AMR ARISHI[1], MOHAMMED ALSHEHRI[1], IBRAHIM MASHHOUR[1]

[1] King Abdulaziz City for Science and Technology (KACST)
P.O. Box 6086 Riyadh 11442, Saudi Arabia

[2] Anadolu University, Faculty of Science, Biology Department, Eskişehir, Turkey

[*]Corresponding author
e-mail: mbokari@kacst.edu.sa
(phone: +966-55-409-0304; fax: +966-11-481-4750)

Abstract
Food-borne pathogens are the leading cause of illness and death in developing countries. Unsafe food storage conditions and poor hygiene practices are major contributing factors to food-associated illnesses in people. Reliable microbiological testing is needed for effective control of food-borne pathogens by the food industry and the food regulation authorities. The aim of this study was to investigate the prevalence of common bacteria in minced meat available in retail shops in Riyadh, Saudi Arabia. A total of 20 different minced meat samples (10 from lamb, 5 from camels and 5 from cows) purchased from local markets in Riyadh were investigated microbiologically. Nine different media (nutrient agar, horse blood agar, sorbitol McConkey agar, chrome agar ECC, chrome agar Salmonella, chrome agar Listeria, chrome agar *Staphylococcus aureus*, chrome agar *Bacillus cereus*, and chrome agar *Escherichia coli* O157) were used to grow and obtain the isolates. Automated riboprinting was applied to the 315 isolates we obtained to assess their genetic similarity levels in the three different food sources. Ribotyping of the total genomic DNA from the strains with *Eco*RI yielded different band patterns. The ribotyping results revealed high genetic diversity among the strains. From the 315 isolates, the Riboprinter detected 240 ribotypes. This is the first study to show the range and number of aerobic bacteria and food-borne contamination in minced meat in Riyadh, using the Riboprinter® Microbial Characterization System.

Key words: Minced meat, Ribotyping, Food-borne bacteria.

Introduction
Red meat is a very nutritious food and a number of animal species (e.g. cows, sheep, goats, camels, deer, horses and pigs) are farmed for it. Food- and water-borne diseases are classified as those that are infectious or toxic following the consumption of contaminated food or water. The common clinical signs and symptoms of such

diseases often take the form of vomiting and diarrhea. Bacteria can contaminate food at any time and the contamination can occur during the slaughter, processing, storage and shipping of the food. Like other foods, red meat has the potential to carry pathogenic organisms to the consumer. The bacterial groups causing food-borne diseases hazardous to health that are present in some meat products include *Salmonella* spp., thermophilic *Campylobacter* spp., enterohemorrhagic *Escherichia coli* (hereafter abbreviated to *Es.* when used with species names), and some serovars of *Yersinia enterocolitica*, *Listeria monocytogenes*, *Clostridium perfringens*, *Staphylococcus* (hereafter abbreviated to *St.* with species names) *St. aureus*, *C. perfringens*, *St. aureus* and *Bacillus cereus* (Roberts et al. 2005; Bhandare et al. 2007; Podpečan et al. 2007). Public health concerns have arisen regarding food-borne illness outbreaks via contamination of food with certain pathogens (e.g., *Salmonella*, *Es. coli* O157: H7, *L. monocytogenes* and others). Infections with these microorganisms can have serious acute impacts and potential chronic long-term complications for people. Some people, such as the elderly, children and immunocompromised individuals are considered at high-risk of contracting such infections (Schmidt and Rodrick 2003). Recent data from developing and developed countries indicate that at least 10% of their populations may experience a food borne disease (Cohen et al. 2006). There are many documented incidents of *Salmonella* occurring in minced meat. Such bacteria were identified in 6.3% of samples (93 samples out of total 1485) of mixed minced meat that was produced in a German slaughter and dissection plant approved by the European Union, where serotyping discovered the presence of *Salmonella typhimurium* (hereafter abbreviated to *Sa.* with species names) in almost 70% of the isolated strains (Stock and Stolle. 2001). It is now known that the cause of the three salmonellosis outbreaks in France between 1998 and 2008 was related to the consumption of minced beef contaminated with *Salmonella*. The salmonella serotypes affecting sheep can potentially lead to food poisoning in humans. Therefore, it is very important to avoid them getting into the food chain. *Sa. havana*, *Sa. anatum* and *Sa. enteritidis* are serotypes especially harmful to sheep, and which cause diarrhoea and death in lambs (Murray, 1984). Significant improvements to the food safety control systems used in many developing countries have been made. However, even in many industrialized countries, food-borne diseases remain a big problem with further work still needed to provide a high level of public health protection from these hazards. It is very important to obtain quantitative and qualitative data on the pathogens in food, and such data needs to be accurate and reliable (Schmidt and Rodrick 2003). In fact, the new techniques used in food control have placed increased emphasis on the process of infection control, and pathogen testing methods remain an integral part of any food producing system, and there is a large market for microbiological tests (Hoorfar 2009). The aim of the present study was to investigate the prevalence of food-borne bacteria such as *Es. coli* O157 H7 and *Salmonella* spp. in raw minced meat samples randomly collected from small butcher shops in Riyadh, Saudi Arabia, where the application of food control systems is less than that in supermarket and governmental slaughterhouses. The Riboprinter® Microbial Characterization System was chosen for use in this study because it is a reliable and powerful tool for the identification and characterization of bacterial isolates (Pavlic and Griffiths 2009).

Materials and methods

Sample collection

A total of 20 minced meat samples from cows (n = 5), camels (n = 5) and sheep (n = 10) were collected from randomly selected retail outlets in Riyadh, Saudi Arabia in November 2016. All the samples were placed in separate sterile plastic bags to prevent cross-contamination and immersed immediately in a packet of ice during transportation to the laboratory.

Isolation of bacteria

From each sample, 25 g was homogenized in 225 mL of distilled water to give an initial 1:10 dilution. Serial dilutions of this first dilution were made and aliquots of 0.1 mL from dilutions up to 10^{-5} were surface plated on sterile dried agar plates. The plates were incubated at 37 °C for 24 h, examined, and then left for another 24 h at room temperature before re-examination. Nine different media (nutrient agar, horse blood agar, sorbitol McConkey agar, chrome agar ECC, chrome agar *Salmonella*, chrome agar *Listeria*, chrome agar *St. aureus*, chrome agar *B. cereus*, chrome agar *Es. coli* O157) were used to grow and obtain the bacterial isolates. All media were purchased from the Saudi Prepared Media Laboratory Company Ltd, Riyadh, Saudi Arabia in pre-prepared petri dishes. Viable cell counts were performed by the spread-plate method after making ten-fold serial dilutions in sterile distilled water as follows: (i) The aerobic total count was conducted on nutrient agar (Oxoid) and horse blood agar incubated at 37 ^{o}C for 24 to 48 h. (ii) Coliforms counts were conducted on chrome agar ECC and sorbitol McConkey agar (Oxoid) incubated at 37 ^{o}C for 24 to 48 h. Typical colonies for chrome agar ECC were blue in colour, while those on sorbitol McConkey agar were round, red-to-pink, 0.5–2 mm in diameter, and surrounded with red-to-pink halos. (iii) Chrome agar *E. coli* (Oxoid) was used to obtain the *Es. coli* counts via incubation at 37 ^{o}C for 18 to 24 h, and the *Es. coli* colonies were typically an intense blue colour. (iv) *St. aureus* colonies on Bio-Rad chrome agar *S. aureus* were incubated at 37 ^{o}C for 24 to 48 h, and the typical colonies were pink in colour. (v) *Bacillus* colonies on chrome agar *B. cereus* at 30 ^{o}C that were blue in colour with a halo were considered positive (vi) *Salmonella* colonies on chrome agar *Salmonella* and, (vii) *Listeria* colonies on chrome agar *Listeria* (Oxoid) were incubated at 37 ^{o}C for 24 to 48 h. In total, 315 presumptive colonies were randomly selected from the different media plates and counted based on their characteristic colony features. The colonies were purified on freshly prepared agar plates that were same as the ones used for their respective isolation. Each colony was then re-streaked onto a specific agar plate for purification. The purified isolates were preliminary characterized by microscopy and Gram reactions. Working cultures were maintained in nutrient broth with 20% glycerol at –80 °C. Moreover, a 0.75 similarity matching score was assigned and used to detect the unique species as shown in Supplementary Tables S1 and S2.

Ribotyping

In this study, automated ribotyping was performed using a robotized instrument (Riboprinter® Microbial Characterization System, Qualicon, Du Pont, Wilmington,

DE, USA) and the DuPont Qualicon database, following the manufacturer's instructions. Briefly, the strains were grown overnight at 37 °C, suspended in buffer from the kit, heated at 80 °C for 10 min and then lysed. The total DNA extracted from them was restricted with *EcoR*I, electrophoretically separated, and then transferred to a membrane for hybridization. A combined dendrogram was prepared from the data using the SPSS statistical program 10.00 (SPSS for Windows Release 10.0 SPSS INC., 1999).

Results

The total number of aerobic bacteria was high in all samples we tested, ranging between $9.8x10^3$ colony forming units (cfu) per g to $4.6x10^4$ cfu/g. The microbiological counts for the minced meat samples are shown in Table 1. *Es. coli* and other coliforms were found in 8/20 samples, *Bacillus* spp. in 2/20 samples, *Staphylococci* in 15/20 samples, while lower numbers (1/20 samples) were obtained for *Listeria* spp. and *Salmonella* spp. *Klebsiella* was found in 3/20 samples. The total plate count and distribution of the bacterial species in the different meat types we studied is shown in Table 1. The highest level of bacterial contamination was observed in the minced meat samples from sheep, with *Staphylococcus* present as the dominant genera.

Table 1. Microbiological counts (log CFU x g-1) detected in minced meat samples.

	Cow		Camel		Sheep	
	Mean	SD	Mean	SD	Mean	SD
Total plate count	2.70	0.51	1.81	0.51	8.01	0.51
Escherichia coli	0.47	0.91	3.26	0.91	3.26	0.91
Coliforms	3.34	0.76	3.96	0.76	3.96	0.76
Staphylococci	6.60	0.15	4.60	0.15	7.60	0.15
Bacillus spp.	2.85	1.04	2.85	1.04	2.85	1.04

A total of 315 isolates were identified in this study and the base line for the similarity matching was set up as ≥ 0.75. As a result of this similarity matching, 180 isolates were considered for species level identification. Distribution of common pathogenic bacteria isolated from minced meat samples ae shown in Fig. 1. However, the bacterial isolates were identified as belonging to 44 genera, of which the *Staphylococcus* genus predominated and was represented by 66 of the 180 isolates. The following *Staphylococci* species and numbers were recorded: *St. aureus* (10), *St. kloosi* (2), *St. saprophyticus* (37), *St. sciuri* (16) and *St. xylosus* (1). Furthermore, 25 isolates of one species of the *Hydrogenophaga* genus (*H.* flava) was identified. The following 17 isolates belonged to the *Enterobacter* genus (hereafter abbreviated to En with species names): *En. aerogenes* (1), *En. cloacae* (15) and *En. gergoviae* (1). *Es. coli* was represented by 17 isolates. Eight isolates were identified in the *Pseudomonas* genus: *Pseudomonas avellanae* (1), *P. fluorescens* (4), and *P. putida* (3). *Serratia* (hereafter abbreviated to *Se.* with species names) were represented by two species, *Se. liquefaciens* and *Se. Marcescens*, of which there were 6 and 1

isolate, respectively. *Variovorax paradoxus* and *Kocuria varians* were detecetd in 5 and 4 samples, respectively.

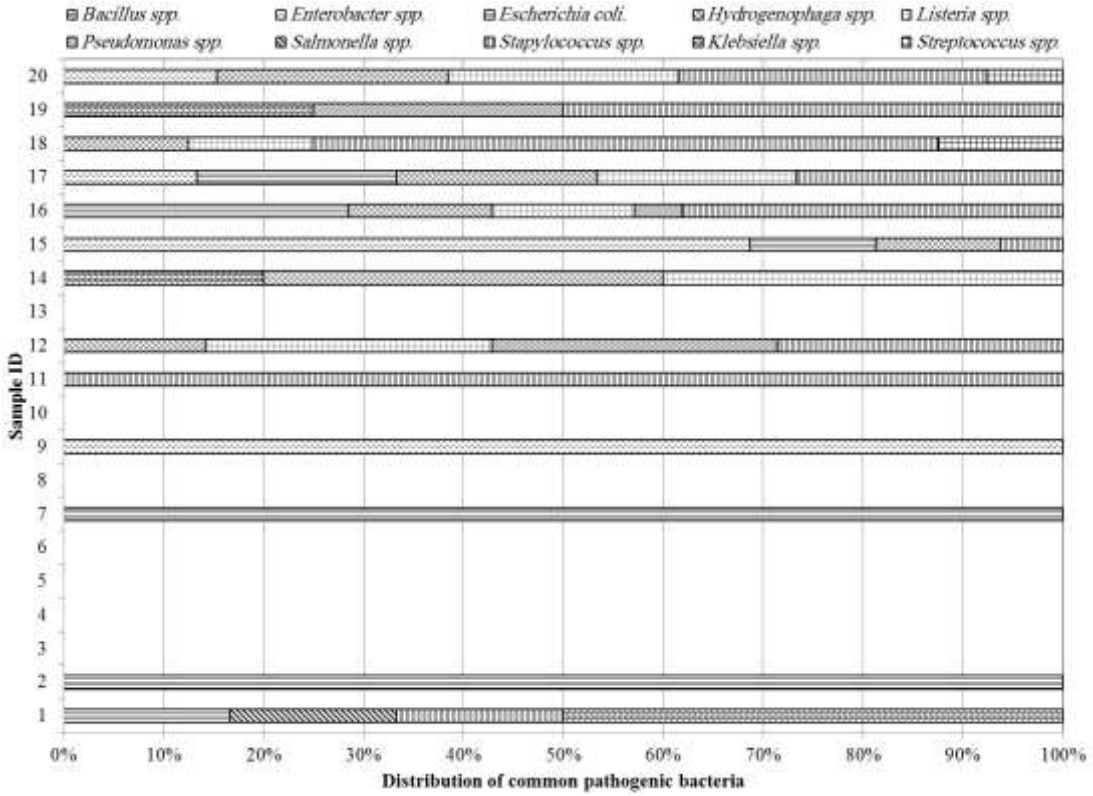

Fig. 1 Frequency distribution of common pathogenic bacteria isolated from minced meat samples.

In addition, 3 isolates were identified each for *Klebsiella pneumoniae*, *Macrococcus caseolyticus*, and *Streptococcus* (hereafter abbreviated to *Str*. with species names). The *Streptococci* isolates, *Str. equiss*, *Str. zooepidemicus* and *Str. sanguinis*, were observed once, but *Str. sanguinis* was observed twice. We also identified one isolate of *Arthrobacter psychrolactophilus* and two isolates of *A. viscosus*. As many as two isolates of other pathogenic bacteria were identified as *Caldicellulosiruptor kristjanssonii*, *L. monocytogenes*, *Oerskovia turbata*, *Pelobacter seleniigenes* and *Bacillus* (*B. insolitus*, *B. licheniformis*). Finally, the remaining species detected were identfied as *Acidovorax temperans*, *Acinetobacter johnsonii*, *Brevibacterium otitidis*, *Brochothrix thermosphacta*, *Celulosirricobium cellulans*, *Citrobacter koseri*, *Delftia acidovorans*, *Micrococcus luteus* and *Sa. havan.*

The Venn diagram (Fig. 2) shows the distribution of bacterial isolates (at the species level) among samples from cows, camels and lamb, with nine different species shared across the samples. Table S3 (Supplementary data) shows the shared species.

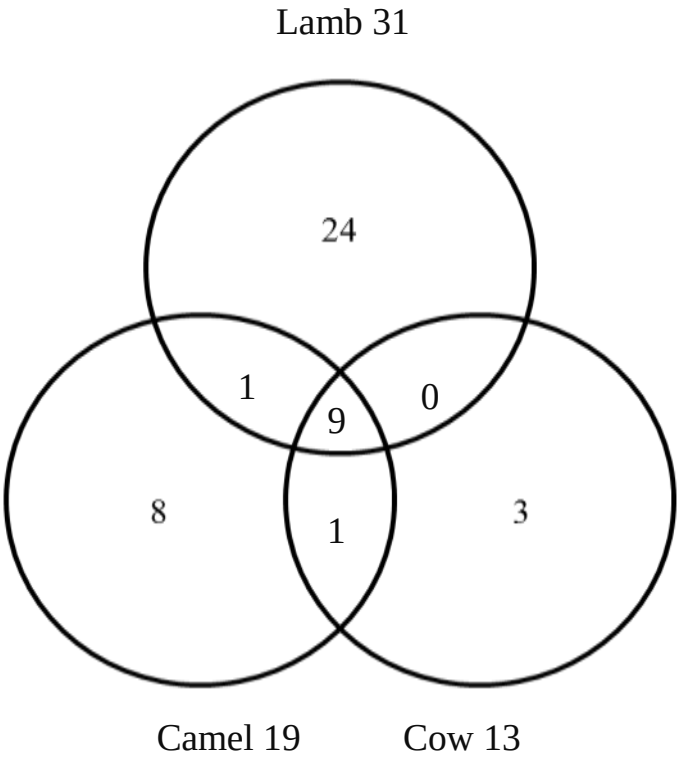

Fig. 2 Venn diagram showing shared species among the meat samples from cows, camels and lamb.

Discussion

Food-borne diseases are widespread and everybody is susceptible to them. However, some factors lead to an increase in the occurrence of such diseases for some people. These factors include the integrity of the immune system, genetic factors, micro and macro nutritional deficiencies and socioeconomic status and they are connected to the individual as well as to their surroundings. According to an estimate from the World Health Organization (WHO), one person out of three in developed countries becomes ill as a result of food-borne pathogens every year. In the USA, it is estimated that approximately 76 million people become infected with food-borne pathogens annually, of which 325,000 instances result in hospitalization and 5,000 in death. Data highlighting the consequences that food contamination and food-borne diseases have on the economy is scarce. These diseases can be caused by food that is not recognized as a potential contamination source before severe illness occurs. Accordingly, any quality control and quality assurance plan must by necessity include an examination protocol for the timely identification of pathogenic microorganisms. Nevertheless, detecting pathogens in food remains challenging because of the high levels of natural bacterial flora (particularly in raw foods), and the unequal distribution the pathogens throughout the food, as well as the range and

complexity of the food types available for consumption. An additional challenge is posed by ingredients that are likely to hinder the reliability and accuracy of bacterial detection assays. Also, food processing can result in increased bacterial numbers (Stevens & Jaykus, 2004; Dwivedi and Jaykus, 2011, WHO). Therefore, testing for the presence and number of specific microorganisms is an integral part of any quality control or quality assurance plan (Torkar el at. 2006). Contemporary methods of food hygiene control focus on, among other things, the control of food processing, and bacterial testing remains an integral part of any system aimed at producing safe food. Currently, according to Hoorfar (2009), the food industry represents the greatest market for microbiological testing. The development of new testing methodology is aimed at forging significant improvements in the risk assessment of food production, and an opportunity exists to model the increasing availability data and information in this area. This approach will enable unreliable data to be discarded. Improved risk assessment models should be able to enhance our understanding of the food production system by analysing the interactions between various parts of the system and assessing the current data and information available about the system. The current safety standards that apply to minced meat samples in Saudi Arabia (Microbiological Criteria for Foodstuffs-Part 1, 1994) are prepared by the Saudi Food and Drug Authority. The guidelines from them mainly target the microbiological quality control of pathogenic bacteria such as *Salmonella, Es. coli, St. aureus and C. perfringens*. This study investigated the presence of pathogenic microorganisms in 20 randomly collected samples of minced meat from sheep (n=10), camels (n=5) and cows (n=5) from the market place. The microbial diversity of viable mesophilic bacteria isolated from the three types of minced meat was revealed by identifying the typical colony morphologies of the organisms based on plating the samples onto specific selective media (commercially known as chromogenic media), followed by Riboprinter biotyping. For colony counting, species isolation and identification, nonselective medium; plate count agar and nutrient agar, which are commonly used to cultivate mesophilic bacteria from food, were employed. Considerable species diversity was found in the samples we investigated, including different genera such as *Pseudomonas* spp., *Staphylococcus* spp. and *Bacillus* spp. We identified 39 strains of *St. saprophyticus* in the meat samples. This result is consistent with the findings of Le Loir (2003); however, we isolated *St. aureus*, a known cause of gastroenteritis, from camel's and cow's meat. Moreover, *St. saprophyticus* is considered to be one of the dominant spoilers of meat (Ercolini et al 1999). *St. aureus* was genetically characterized and isolated from retail meat in Riyadh, Saudi Arabia (Raji et al , 2016). Although *Salmonella* spp. and *Listeria* spp. should be undetectable in a 25-g meat sample according to regulations (Saudi FDA, p9 –10, 1994), the *Sa. havana* isolate we examined was from camel (sample no. 1) while a *L. monocytogenes* isolate was detected in a sample from sheep (sample no. 12). The former can cause severe life-threatening disease while the latter can also be very dangerous; both isolates are considered food-borne pathogens capable of causing serious sickness, especially in the elderly and in immunocompromised patients, pregnant women, newborns and infants (Rebagliati et al. 2009). These species were mentioned in a food-borne illness surveillance study (Al-Goblan and Jahan, 2010) carried out on the data collocation for 2006 in the

Qassim province of Saudi Arabia. The study dealt with 31 food-borne illness outbreaks consisting of 251 individual recorded cases. Salmonella food poisoning outbreaks have been reported in Saudi Arabia since 1984 and according to Aljoudi et al (2010), there were 211.6 cases per month. Salmonella serotypes can be highly pathogenic and infected sheep should be kept isolated because bacteria like *Sa. anatum, Sa. enteritidis* and *Sa. havana* have led to deaths in lambs (Aljoudi et.al, 2010). Contamination of two camel samples (Nos. 1 and 2), one cow sample (No. 7) and three sheep samples (Nos. 15, 16 and 17) with *Es. coli* meant that these samples failed food safety standards testing (Saudi FDA, p 9–10, 1994). The presence of these bacteria in the samples indicates faecal contamination and the potential presence of other dangerous pathogens. Consumption of food that contains $>10^5$–10^6 *B. cereus*/g may result in food poisoning (Dierick et al. 2005). According to our results, only two lamb samples (Nos. 14 and 19) were contaminated with *B. insolitus* and *B. licheniformis*. Microbiological contamination of minced meat can be of primary or secondary origin. For example, the microorganisms present in animal tissues before slaughter constitute primary contaminants, whereas the secondary ones occur during the course of product manufacturing, storage or distribution. The initial level of post-productive contamination, as well as the numerous intrinsic and extrinsic parameters of the product itself will determine its microbiological stability and, consequently, the safety of the consumer (Nørrung el at. 2008).

Conclusion

Identification of pathogenic bacteria for food quality assurance becomes very necessary due to different sources of food, especially meat. In this study, 20 randomly collected samples showed that only a one third of the samples contained non-pathogenic bacteria, while the rest contained various quantities and types of pathogenic bacteria. Staphylococcus spp. were dominant in as many as five samples, while Enterobacter spp., Es. coli, Hydrogenophaga spp. and Listeria spp. were present in multiple samples. Salmonella spp. and Klebsiella spp. were identified in a single sample. The lamb samples were richer in bacteria than those from camels and cows, and nine species were shared among the three sources of meat. Expanding cities and increasing numbers of shops in Saudi Arabia created a pressing need to apply a fast and reliable technique to identify foodborne pathogens, especially in minced meat because of the high possibility of its contamination during processing.

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Supplementary Table S1: Similarity matching more than 0.75

Genus	Dupont ID Label	Isolates	Dupont ID Similarity
Acidovorax	*Acidovorax temperens*	1	0.81
Acinetobacter	*Acinetobacter johnsonii*	1	0.83
Arthrobacter	*Arthrobacter psychrolactophilus*	3	0.77
	Arthrobacter viscosus		0.75
	Arthrobacter viscosus		0.82
Bacillus	*Bacillus insolitus*	1	0.76
	Bacillus licheniformis	1	0.75
Brevibacterium	*Brevibacterium otitidis*	1	0.83
Brochothrix	*Brochothrix thermosphacta*	1	0.88
Caldicellulosiruptor	*Caldicellulosiruptor kristjanssonii*	2	0.82
	Caldicellulosiruptor kristjanssonii		0.87
Celulosirricobium	*Celulosirricobium cellulans*	1	0.83
Citrobacter	*Citrobacter koseri*	1	0.75
Delftia	*Delftia acidovorans*	1	0.87
Enterobacter	*Enterobacter aerogenes*	17	0.75
	Enterobacter cloacae		0.75
	Enterobacter cloacae		0.76
	Enterobacter cloacae		0.76
	Enterobacter cloacae		0.76
	Enterobacter cloacae		0.77
	Enterobacter cloacae		0.79
	Enterobacter cloacae		0.79
	Enterobacter cloacae		0.79
	Enterobacter cloacae		0.8
	Enterobacter cloacae		0.81
	Enterobacter cloacae		0.83
	Enterobacter cloacae		0.85
	Enterobacter cloacae		0.87
	Enterobacter cloacae		0.88
	Enterobacter cloacae		0.9
	Enterobacter gergoviae		0.82
Escherichia	*Escherichia coli*	17	0.8
	Escherichia coli		0.82
	Escherichia coli		0.83
	Escherichia coli		0.84
	Escherichia coli		0.84

Genus	Dupont ID Label	Isolates	Dupont ID Similarity
	Escherichia coli		0.84
	Escherichia coli		0.87
	Escherichia coli		0.88
	Escherichia coli		0.88
	Escherichia coli		0.9
	Escherichia coli		0.9
	Escherichia coli		0.92
	Escherichia coli		0.92
	Escherichia coli		0.93
	Escherichia coli		0.94
	Escherichia coli		0.96
	Escherichia coli		0.96
	Hydrogenophaga flava		0.75
	Hydrogenophaga flava		0.76
	Hydrogenophaga flava		0.76
	Hydrogenophaga flava		0.78
	Hydrogenophaga flava		0.78
	Hydrogenophaga flava		0.79
	Hydrogenophaga flava		0.79
	Hydrogenophaga flava		0.79
	Hydrogenophaga flava		0.79
	Hydrogenophaga flava		0.8
	Hydrogenophaga flava		0.8
	Hydrogenophaga flava		0.8
Hydrogenophaga	*Hydrogenophaga flava*	25	0.8
	Hydrogenophaga flava		0.8
	Hydrogenophaga flava		0.8
	Hydrogenophaga flava		0.8
	Hydrogenophaga flava		0.8
	Hydrogenophaga flava		0.81
	Hydrogenophaga flava		0.81
	Hydrogenophaga flava		0.81
	Hydrogenophaga flava		0.81
	Hydrogenophaga flava		0.83
	Hydrogenophaga flava		0.83
	Hydrogenophaga flava		0.85
	Hydrogenophaga flava		0.9

Genus	Dupont ID Label	Isolates	Dupont ID Similarity
Klebsiella	*Klebsiella pneumoniae*	3	0.82
	Klebsiella pneumoniae		0.83
	Klebsiella pneumoniae		0.91
Kocuria	*Kocuria varians*	4	0.75
	Kocuria varians		0.75
	Kocuria varians		0.81
	Kocuria varians		0.81
Lactobacillus	*Lactobacillus paracasei ss. Paracasei*		0.78
Listeria	*Listeria monocytogenes*	2	0.78
	Listeria monocytogenes		0.81
Macrococcus	*Macrococcus caseolyticus*	3	0.78
	Macrococcus caseolyticus		0.83
	Macrococcus caseolyticus		0,71
Micrococcus	*Micrococcus luteus*	1	0.83
Oerskovia	*Oerskovia turbata*	2	0.75
	Oerskovia turbata		0.77
Pelobacter	*Pelobacter seleniigenes*	2	0.78
	Pelobacter seleniigenes		0.78
Pseudomonas	*Pseudomonas avellanae*	8	0.84
	Pseudomonas fluorescens		0.78
	Pseudomonas fluorescens		0.85
	Pseudomonas fluorescens		0.86
	Pseudomonas fluorescens		0.91
	Pseudomonas putida		0.89
	Pseudomonas putida		0.95
	Pseudomonas putida		0.96
Salmonella	*Salmonella ser. Havana*	1	0.85
Serratia	*Serratia liquefaciens*	7	0.79
	Serratia liquefaciens		0.85
	Serratia liquefaciens		0.89
	Serratia liquefaciens		0.91
	Serratia liquefaciens		0.91
	Serratia liquefaciens		0.95
	Serratia marcescens		0.91
Staphylococcus	*Staphylococcus aureus*	66	0.75
	Staphylococcus aureus		0.77
	Staphylococcus aureus		0.78

Genus	Dupont ID Label	Isolates	Dupont ID Similarity
	Staphylococcus aureus		0.79
	Staphylococcus aureus		0.8
	Staphylococcus aureus		0.8
	Staphylococcus aureus		0.84
	Staphylococcus aureus		0.85
	Staphylococcus aureus		0.88
	Staphylococcus aureus		0.89
	Staphylococcus kloosii		0.87
	Staphylococcus kloosii		0.87
	Staphylococcus saprophyticus		0.76
	Staphylococcus saprophyticus		0.77
	Staphylococcus saprophyticus		0.77
	Staphylococcus saprophyticus		0.78
	Staphylococcus saprophyticus		0.79
	Staphylococcus saprophyticus		0.82
	Staphylococcus saprophyticus		0.82
	Staphylococcus saprophyticus		0.83
	Staphylococcus saprophyticus		0.83
	Staphylococcus saprophyticus		0.83
	Staphylococcus saprophyticus		0.84
	Staphylococcus saprophyticus		0.85
	Staphylococcus saprophyticus		0.85
	Staphylococcus saprophyticus		0.86
	Staphylococcus saprophyticus		0.86
	Staphylococcus saprophyticus		0.87
	Staphylococcus saprophyticus		0.88
	Staphylococcus saprophyticus		0.88
	Staphylococcus saprophyticus		0.89
	Staphylococcus saprophyticus		0.89
	Staphylococcus saprophyticus		0.89
	Staphylococcus saprophyticus		0.9
	Staphylococcus saprophyticus		0.91
	Staphylococcus saprophyticus		0.92
	Staphylococcus saprophyticus		0.92
	Staphylococcus saprophyticus		0.92
	Staphylococcus saprophyticus		0.92
	Staphylococcus saprophyticus		0.93

Genus	Dupont ID Label	Isolates	Dupont ID Similarity
	Staphylococcus saprophyticus		0.93
	Staphylococcus saprophyticus		0.94
	Staphylococcus saprophyticus		0.94
	Staphylococcus saprophyticus		0.95
	Staphylococcus saprophyticus		0.95
	Staphylococcus saprophyticus		0.95
	Staphylococcus saprophyticus		0.95
	Staphylococcus saprophyticus		0.96
	Staphylococcus saprophyticus		0.94
	Staphylococcus sciuri		0.76
	Staphylococcus sciuri		0.76
	Staphylococcus sciuri		0.78
	Staphylococcus sciuri		0.8
	Staphylococcus sciuri		0.86
	Staphylococcus sciuri		0.87
	Staphylococcus sciuri		0.87
	Staphylococcus sciuri		0.88
	Staphylococcus sciuri		0.88
	Staphylococcus sciuri		0.88
	Staphylococcus sciuri		0.89
	Staphylococcus sciuri		0.9
	Staphylococcus sciuri		0.91
	Staphylococcus sciuri		0.91
	Staphylococcus sciuri		0.94
	Staphylococcus sciuri		0.96
	Staphylococcus xylosus		0.78
Streptococcus	*Streptococcus equi ss. zooepidemicus*	3	0.79
	Streptococcus sanguinis		0.75
	Streptococcus sanguinis		0.76
Variovorax	*Variovorax paradoxus*	5	0.81
	Variovorax paradoxus		0.84
	Variovorax paradoxus		0.85
	Variovorax paradoxus		0.88
	Variovorax paradoxus		0.89

Supplementary Table S2: Similarity matching less than 0.75

Genus	Dupont ID Label	Isolates	Dupont ID Similarity
Acinetobacter	*Acinetobacter johnsonii*	*5*	0.67
	Acinetobacter lwoffii		0.66
	Acinetobacter lwoffii		0.69
	Acinetobacter lwoffii		0.72
	Acinetobacter species		0.74
Aeromonas	*Aeromonas media*	*7*	0.68
	Aeromonas media		0.71
	Aeromonas salmonicida ss. achromogenes		0.7
	Aeromonas salmonicida ss. achromogenes		0.7
	Aeromonas salmonicida ss. achromogenes		0.72
	Aeromonas salmonicida ss. achromogenes		0.73
	Aeromonas veronii		0.63
Alicyclobacillus	*Alicyclobacillus acidocaldarius*	*1*	0.65
Atopobium	*Atopobium parvulum*	*2*	0.72
	Atopobium rimae		0.63
Azohydromonas	*Azohydromonas lata*	*1*	0.73
Bacillus	*Bacillus alcalophilus*	*11*	0.47
	Bacillus cereus		0.63
	Bacillus clausii		0.58
	Bacillus clausii		0.6
	Bacillus clausii		0.63
	Bacillus coagulans		0.63
	Bacillus horikoshii		0.62
	Bacillus lentus		0.65
	Bacillus mojavensis		0.58
	Bacillus pseudofirmus		0.71
	Bacillus species		0.66
Bifidobacterium	*Bifidobacterium animalis*	*1*	0.74
Brevibacillus	*Brevibacillus agri*	*2*	0.63
	Brevibacillus brevis		0.65
Brevibacterium	*Brevibacterium mcbrellneri*	*1*	0.71
Chryseobacterium	*Chryseobacterium indologenes*	*1*	0.72
Citrobacter	*Citrobacter freundii*	*2*	0.59
	Ctrobacter freundii		0.71
Delftia	*Delftia acidovorans*	*1*	0.61
Enterobacter	*Enterobacter aerogenes*	*9*	0.71
	Enterobacter aerogenes		0.73
	Enterobacter cloacae		0.61
	Enterobacter cloacae		0.69
	Enterobacter cloacae		0.7
	Enterobacter hormaechei		0.63
	Enterobacter sakazakii		0.61
	Enterobacter sakazakii (Cronobacter species)		0.61
	Enterobacter sakazakii (Cronobacter species)		0.62
Enterococcus	*Enterococcus avium*	*1*	0.59
Escherichia	*Escherichia coli*	*2*	0.57

	Escherichia coli		0.6
Geobacillus	*Geobacillus thermoleovorans*	*1*	0.53
Hydrogenophaga	*Hydrogenophaga flava*	*13*	0.54
	Hydrogenophaga flava		0.64
	Hydrogenophaga flava		0.65
	Hydrogenophaga flava		0.66
	Hydrogenophaga flava		0.67
	Hydrogenophaga flava		0.68
	Hydrogenophaga flava		0.69
	Hydrogenophaga flava		0.69
	Hydrogenophaga flava		0.71
	Hydrogenophaga flava		0.71
	Hydrogenophaga flava		0.74
	Hydrogenophaga flava		0.74
	Hydrogenophaga flava		0.74
Klebsiella	*Klebsiella pneumoniae*	*2*	0.64
	Klebsiella pneumoniae		0.66
Kocuria	*Kocuria rosea*	*2*	0.65
	Kocuria varians		0.55
Lactobacillus	*Lactobacillus helveticus*	*1*	0.64
Lactococcus	*Lactococcus plantarum*	*1*	0.54
Leclercia	*Leclercia adecarboxylata*	*1*	0.68
Macrococcus	*Macrococcus caseolyticus*	*7*	0.59
	Macrococcus caseolyticus		0.62
	Macrococcus caseolyticus		0.71
	Macrococcus caseolyticus		0.72
	Macrococcus caseolyticus		0.72
	Macrococcus species		0.6
	Macrococcus species		0.62
Moraxella	*Moraxella osloensis*	*2*	0.69
	Morganella morganii		0.72
Paenibacillus	*Paenibacillus species*	*1*	0.7
Pseudomonas	*Pseudomonas aeruginosa*	*3*	0.56
	Pseudomonas fluorescens		0.68
	Pseudomonas putida		0.61
Rothia	*Rothia mucilaginosa*	*1*	0.66
Salmonella	*Salmonella ser. Ealing*	*2*	0.64
	Salmonella ser. Ealing		0.68
Sphingomonas	*Sphingomonas species*	*1*	0.58
Staphylococcus	*Staphylococcus arettae*	*38*	0.6
	Staphylococcus aureus		0.47
	Staphylococcus aureus		0.59
	Staphylococcus aureus		0.61
	Staphylococcus aureus		0.62
	Staphylococcus aureus		0.64
	Staphylococcus aureus		0.64
	Staphylococcus aureus		0.64
	Staphylococcus aureus		0.65
	Staphylococcus aureus		0.65
	Staphylococcus aureus		0.66
	Staphylococcus aureus		0.66

	Staphylococcus aureus		0.66
	Staphylococcus aureus		0.67
	Staphylococcus aureus		0.67
	Staphylococcus aureus		0.68
	Staphylococcus aureus		0.69
	Staphylococcus aureus		0.69
	Staphylococcus aureus		0.69
	Staphylococcus aureus		0.7
	Staphylococcus aureus		0.7
	Staphylococcus aureus		0.73
	Staphylococcus aureus		0.74
	Staphylococcus capitis		0.61
	Staphylococcus capitis		0.63
	Staphylococcus capitis		0.64
	Staphylococcus epidermidis		0.6
	Staphylococcus equorum		0.65
	Staphylococcus saprophyticus		0.64
	Staphylococcus saprophyticus		0.74
	Staphylococcus saprophyticus		0.74
	Staphylococcus sciuri		0.67
	Staphylococcus sciuri		0.67
	Staphylococcus sciuri		0.7
	Staphylococcus vitulinus		0.67
	Staphylococcus warneri		0.74
	Staphylococcus xylosus		0.67
	Staphylococcus xylosus		0.74
Streptococcus	*Streptococcus agalactiae*	*8*	0.68
	Streptococcus parasanguinis		0.58
	Streptococcus pneumoniae		0.72
	Streptococcus pneumoniae		0.74
	Streptococcus thermophilus		0.74
	Streptococcus uberis		0.63
	Streptococcus uberis		0.64
	Streptococcus uberis		0.65
Tindallia	*Tindallia californiensis*	*1*	0.71
Vibrio	*Vibrio parahaemolyticus*	*2*	0.65
	Vibrio species		0.71

Supplementary Table S3: Sharing species Venn Diagram

Common Species	Sheep & Camel	Sheep & Cow	Camel & Cow	Camel Only	Cow Only	Sheep Only
Escherichia coli	*staphylococcus saprophyticus*	*Pseudomonas fluorescens*	*pseudomonas putida*	*Azohydromonas lata*	*lactobacillus paracasei*	*Delftia acidovorans*
Hydrogenophaga flava	*Pseudomonas fluorescens*	*Hydrogenophaga flava*	*staphylococcus saprophytic*	*Staphylococcus xylosus*	*Enterobacter aerogenes*	*Acidovorax temperans*
Pseudomonas fluorescens	*Staphylococcus saprophytic*	*Escherichia coli*	*Hydrogenophaga flava*	*Arthrobacter psychrolactophilus*	*Staphylococcus aureus*	*Pelobacter seleniigenes*
Serratia liquefaciens	*Hydrogenophaga flava*	*pseudomonas putida*	*Escherichia coli*	*Oerskovia turbata*		*kocuria varians*
Staphylococcus saprophyticus	*variovorax paradoxus*	*variovorax paradoxus*	*Pseudomonas fluorescens*	*Brevibacterium otitidis*		*Enterobacter cloacae*
Staphylococcus saprophytic	*Macrococcus caseolyticus*	*Serratia liquefaciens*	*Serratia liquefaciens*	*caldicellulosiruptor kristjanssonii*		*Citrobacter koseri*
Variovorax paradoxus	*Staphylococcus sciuri*	*Staphylococcus sciuri*	*staphylococcus saprophyticus*	*Salmonella ser. Havana*		*Acinetobacter johnsonii*
	Serratia liquefaciens	*staphylococcus saprophyticus*	*Variovorax paradoxus*	*klebsiella pneumoniae*		*Gluconobacter serinus*
	variovorax paradoxus	*Staphylococcus saprophytic*				*gluconobacter diazotrophicus*
						Acinetobacter lwoffii
						arthrobacter viscosus
						staphylococcus kloosii
						Serratia marcescen
						Enterobacter gergoviae
						Brochothrix thermosphacta
						Streptococcus equi ss. Zooepidemi
						pseudomonas avellanae
						Gluconobacter oxydans

						Thermus igniterrae
						micrococcus luteus
						Cellulosimicrobium cellulans
						Streptococcus sanguis
						Streptococcus sanguinis
						Listeria monocytogenes