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Source: Avian Diseases, 67(1) : 1-9

Published By: American Association of Avian Pathologists

URL: https://doi.org/10.1637/aviandiseases-D-22-00048

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AVIAN DISEASES 67:1–9, 2023

Review Article—

Microbiota of Chickens and Their Environment in Commercial Production

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Received 30 October 2022; Accepted 4 November 2022; Published ahead of print 19 January 2023

SUMMARY. Chickens in commercial production are subjected to constant interaction with their environment, including the exchange of microbiota. In this review, we therefore focused on microbiota composition in different niches along the whole line of chicken production. We included a comparison of microbiota of intact eggshells, eggshell waste from hatcheries, bedding, drinking water, feed, litter, poultry house air and chicken skin, trachea, crop, small intestine, and cecum. Such a comparison showed the most frequent interactions and allowed for the identification of microbiota members that are the most characteristic for each type of sample as well as those that are the most widespread in chicken production. Not surprisingly, *Escherichia coli* was the most widely distributed species in chicken production, although its dominance was in the external aerobic environment and not in the intestinal tract. Other broadly distributed species included *Ruminococcus torque*, *Clostridium disporicum*, and different *Lactobacillus* species. The consequence and meaning of these and other observations are evaluated and discussed.

RESUMEN. *Estudio recapitulativo-* Microbiota de pollos y su entorno en producción comercial.

Los pollos en producción comercial están sujetos a una interacción constante con su entorno, incluido el intercambio de microbiota. Esta revisión, por lo tanto, se enfoca en la composición de la microbiota en diferentes nichos a lo largo de toda la línea de producción de pollos. Se incluye una comparación de microbiota de cascarones de huevo intactos, desechos de cascarones de huevos de plantas de incubación, cama, agua potable, alimento, cama, aire de gallinero y piel de pollo, tráquea, buche, intestino delgado y ciego. Tal comparación mostró las interacciones más frecuentes y permitió identificar los miembros de la microbiota más característicos para cada tipo de muestra, así como los más extendidos en la producción de pollos. No en vano, *Escherichia. coli* fue la especie más ampliamente distribuida en la producción de pollos, aunque su dominio fue en el ambiente aeróbico externo y no en el tracto intestinal. Otras especies ampliamente distribuidas incluyeron *Ruminococcus torque*, *Clostridium disporicum* y diferentes especies de *Lactobacillus*. Se evalúan y discuten la consecuencia y el significado de estas y otras observaciones.

Key words: chicken, microbiota, environment, litter, bedding, feed, cecum, crop

Abbreviations: rRNA = ribosomal RNA; OTU = operational taxonomic unit; CFU = colony-forming units

Every live animal is in constant interaction with its environment. It eats, drinks, and breathes, and it also excretes end products of its metabolism into the environment. The whole surface of animals is exposed to and is in interaction with solid, liquid, and gaseous environments. Because we do not live in a sterile world, these interactions are inevitably also associated with an exchange of complex microbial populations. Humans or animals are colonized by bacteria from different types of environments, including those with a negative effect on animal performance. How these individual environments are similar or dissimilar in terms of microbial composition becomes increasingly understood due to developments in nucleic acid sequencing. However, different compartments are usually analyzed separately and only a few papers comparing microbiota composition in chickens and their abiotic environment have been published (1,2,3,4).

In this review, we therefore evaluated the total environment in chicken production by comparing microbiota composition at key production points, thus attempting to define likely transmissions and microbiota members characteristic for each environment. To reach this aim, we first used data from our own studies on intact eggshells sampled prior to cleaning and disinfection in commercial hatchery, feed, crop, and gut microbiota (5,6,7,8,9,10). To bridge missing information, this dataset was enriched with yet-unpublished samples of litter, remains of eggshells in hatcheries, drinking water, and chicken skin and trachea (Fig. 1, see Supplemental Information S1 for analysis protocol and Supplemental Table S2 for a list of samples). This comparison was used as an introduction to the topic that was then developed with published data. To minimize bias introduced by selective culture, information provided in this review is based mainly on sequencing data. This approach also has its drawbacks. First, using sequencing data, we lost information on absolute bacterial counts. Cecal microbiota reach 10^9 colony-forming units (CFU)/g of digesta, microbiota in the small intestine is present at approximately 10^6 CFU/g of digesta, and feed may contain around 10^3 CFU/g. Despite this difference in CFU, percentage abundances result in a similar visual impression. Second, sequencing detects DNA from both viable and nonviable bacteria. This can be important for detecting microbiota in clean bedding, fresh feed, or cecal anaerobes in the litter where an increased number of nonviable bacteria can be expected. The latter point we treated with an approach in which we concentrated on major representatives present in different samples for which it is rather difficult to imagine that they can reach considerable abundance without being viable.

Initial analysis of selected samples. When a global composition at the phylum level was compared with that from our previous studies (5,6,7,8,9,10) and unpublished data to overcome missing data (see Supplemental Table S2 and Supplemental Table S3 for the type and number of samples, sequence coverage, and microbiota composition), aerobic compartments associated with chickens were enriched for Gram-positive Firmicutes. Litter was characterized by the presence of Gram-positive Actinobacteria and Firmicutes; however,

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Fig. 1. Types of samples from poultry production covered in this review. Microbiota composition was determined and compared in different chicken and environmental compartments.

litter Firmicutes were different from Firmicutes colonizing chickens directly. Firmicutes in the litter comprised mainly *Staphylococcaceae*, whereas Firmicutes associated with chickens included families *Lachnospiraceae*, *Ruminococcaceae*, *Oscillospiraceae*, *Acidaminococcaceae*, and *Selenomonadaceae*. The anaerobic cecum was colonized by Firmicutes and Bacteroidetes, and environmental samples were colonized mostly by Gram-negative Proteobacteria and Bacteroidetes. However, environmental Bacteroidetes were different from those in the cecum (Fig. 2 and see below). Cecal Bacteroidetes belonged to class Bacteroidia, whereas environmental Bacteroidetes belonged mostly to Flavobacteria and Sphingobacteria.

Principal component analysis using Unifrac distances showed a clear separation of cecal and feed microbiota from the rest of the samples. Clean bedding and drinking water microbiota also formed separated clusters, indicating specific microbiota compositions. Tracheal and skin microbiota samples overlapped, and samples from the crop and ileum were also similar. Litter microbiota clustered close to biological samples from the chicken, likely due to constant interactions. Microbiota from eggshell waste formed a separate cluster, indicating specific microbiota composition, whereas microbiota from eggshells from intact eggs clustered between cecal and litter samples, suggesting that at least some microbiota members were shared by these three sample types (Fig. 3).

However, are these predictions in agreement with published data? What are the species characteristics for each ecological niche? Are there any species that can be found across more samples, despite overall sample dissimilarity, thus representing chicken panmicrobiota members? All of these questions are reviewed below.

Intact eggshell microbiota. The eggshell microbiota is mainly a combination of gut and litter microbiota. The eggshell microbiota is formed by Firmicutes, Bacteroidetes, and Actinobacteria, whereas Proteobacteria are usually underrepresented (5,11,12). The most

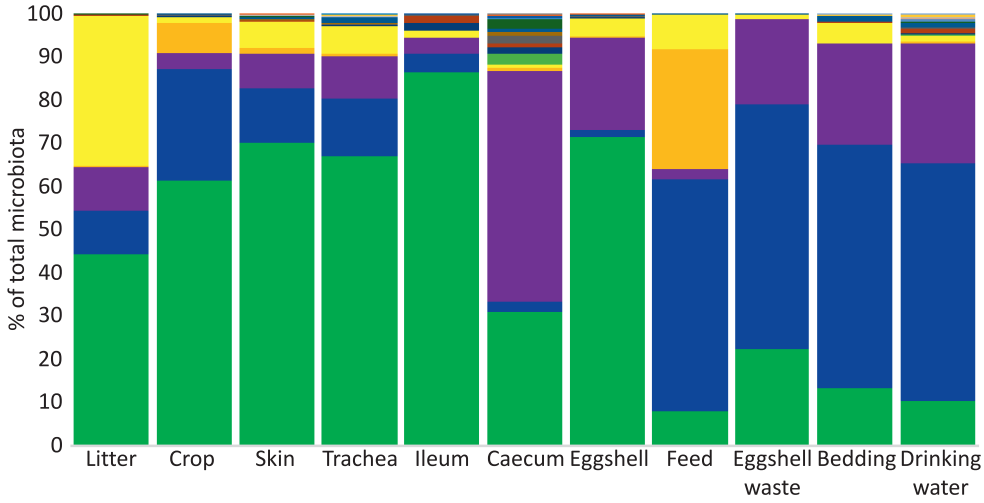

Fig. 2. Composition of chicken microbiota and their environment at phylum level. Yellow = Actinobacteria, magenta = Bacteroidetes, blue = Proteobacteria, green = Firmicutes, orange = Cyanobacteria. Cyanobacteria sequences originated from plant chloroplast rRNA, and this is why these signatures are present in the feed samples and crop where the feed is not completely digested yet. Please see Supplemental Table S3 for the identification of all cecal microbiota OTUs.

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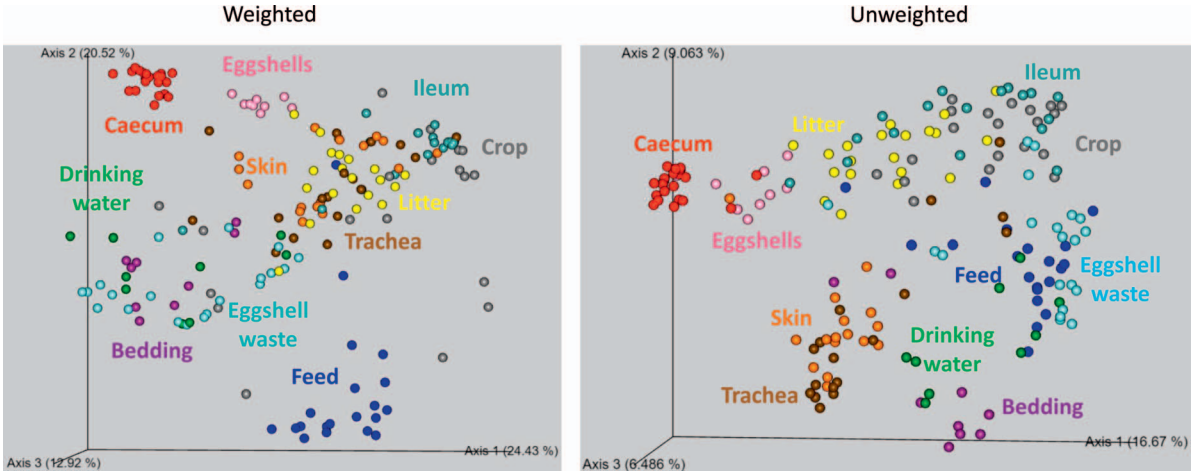

Fig. 3. Microbiota in the whole production system of chickens. Cecal microbiota differed from the rest of samples. Ileum and crop microbiota overlapped, as well as skin and respiratory tract microbiota. Litter microbiota was always close to eggshell microbiota, and eggshell microbiota clustered close to the cecal microbiota.

common genera of those originating from the intestinal tract include *Lactobacillus*, *Bacteroides*, *Megamonas*, *Olsenella*, *Faecalibacterium*, *Oscillibacter*, *Clostridium*, *Pseudoflavonifractor*, *Blautia*, or *Ruminococcus* (5,11,12,13). These bacteria are deposited on the surface of eggs during egg lay, as a consequence of fecal contamination. However, that they embed into the forming eggshell as early as in the reproductive tract cannot be excluded, as fecal microbiota members can also be found in the reproductive tract (14). Despite the presence of fecal microbiota on the eggshells, these bacteria do not survive cleaning and disinfection together with egg incubation and do not appear in gut microbiota of hatched chickens (5). Indirect evidence of no transfer of eggshell microbiota to chicken gut microbiota can also be deduced from the comparison of *Bacteroides*, *Megamonas*, or *Olsenella* presence on eggshell but their rare presence in gut microbiota of chicks during first week of life (8,15,16).

Litter also contributes to the eggshell microbiota. Bacterial genera of environmental origin, such as *Brachybacterium*, *Brevibacterium*, *Corynebacterium* (all Actinobacteria), *Staphylococcus*, *Salinicoccus*, *Yaniella*, *Jeotgalicoccus*, *Aerococcus* (all Firmicutes), or *Pseudomonas* (Proteobacteria), were repeatedly detected on eggshells (5,11,12,13). These bacteria are usually less abundant than those of intestinal origin (5), but this difference may vary depending on the extent of egg contamination by fecal and litter material. The presence of *Acinetobacter*, *Streptococcus*, and *Enterococcus* on eggshells was reported less frequently (5,11,13). Due to aerotolerance, these species are likely viable on the eggshells because, unlike all other eggshell microbiota, they appear also in eggshell waste when the hatching is complete (see below).

Eggshell waste microbiota. There are no published data on microbiota composition in eggshell waste despite the fact that bacteria in the eggshell waste are the first ones to which newly hatched chicks are exposed. In a preliminary way, we analyzed 20 different samples of this type collected from the floor of a hatching incubator when the hatching was accomplished, and chicks were already deployed (Supplemental Table S2). The most common bacteria included *Brevibacterium* (Actinobacteria); *Chryseobacterium* and *Flavobacterium* (Bacteroidetes); *Enterococcus*, *Clostridium paraputrificum*, and *Clostridium disporicum* (Firmicutes); and *Acinetobacter*, *Comamonas*, *Enterobacter*, *Escherichia*, *Proteus*, and *Pseudomonas* (all Gammaproteobacteria) (Supplemental Table S3). Of these bacteria, *Acinetobacter*, *Chryseobacterium*, *C. disporicum*, *Escherichia*, *Pseudomonas*, *Comamonas*, and *Enterococcus* were among the most abundant ones in the waste from hatcheries. These bacteria then appeared in the litter during the first days after chicken placement on the farm but did not belong to dominant and permanent litter microbiota members. This finding is true also for *Brevibacterium* present in eggshell waste that belonged to *Brevibacterium luteolum*, whereas those present in the litter belonged to species *Brevibacterium iodinum* or *Brevibacterium senegalense*. Similarly, except for *Escherichia coli*, bacteria from eggshell waste did not enter and colonize the chicken intestinal tract, most probably due to the absence of efficient anaerobic metabolism. Although these bacteria did not colonize the chicken intestinal tract, they may cause early activation of the chicken immune system, or some of them may suppress the multiplication of pathogenic bacteria like *Salmonella* in the hatcheries, thus decreasing the introduction of this pathogen into the flocks, similar to the approach of litter treatment by *Bacillus* spores (17).

Drinking water microbiota. Although different biologically active products like probiotic lactobacilli or bacteriophages are distributed by drinking water in chicken production (18,19), we did not find a report that classified the microbiota composition in drinking water from poultry houses. This is quite surprising because an aquatic environment supports the survival of bacteria including pathogenic ones and chickens continuously consume drinking water. Our analysis of nine samples of drinking water from four different animal houses (including automatic drinking system from broiler farms as well as daily replaced water containers from experimental animal house, see Supplemental Table S2) is therefore the first attempt and should stimulate further research. Drinking water microbiota from one-week-old flocks was quite diverse and differed in individual samples. Unlike other types of samples, drinking water samples were colonized by a few genera, with each of them forming around 5%–10% of the tested sample microbiota. Drinking water contained mostly Gammaproteobacteria and genera *Escherichia*, *Acinetobacter*, and *Pseudomonas*. Additional taxa included genera *Flavobacterium*, *Chryseobacterium* and *Sphingobacterium* (phylum Bacteroidetes), and *Sulfurospirillum* (phylum Campylobacterota, earlier Epsilonproteobacteria). Firmicutes in drinking water were represented by *Lactobacillus aviarius* and *Veillonella magna*. Alphaproteobacteria

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found in drinking water (genera *Brevundimonas*, *Caulobacter*, *Novosphingobium*, or *Sphingomonas*) were detected earlier in the intestinal tract of chickens as apparently difficult to culture (20). It is possible that they are aerobic bacteria incapable of anaerobic multiplication that only passively enter the chicken intestinal tract via drinking water. Because anaerobic culture was used by Crhanova *et al.* (20), it may explain the inability to culture these bacteria. The same operational taxonomic units (OTUs) as those in drinking water were recorded in waste from eggshells (*Sphingobacterium*, *Escherichia*, and *Acinetobacter*), the skin (*L. aviarius*, *V. magna*, *Brevundimonas*, *Novosphingobium*, *Sphingomonas*, *Escherichia*, *Acinetobacter*, and *Pseudomonas*), and the trachea (*L. aviarius*, *V. magna*, *Novosphingobium*, *Sphingomonas*, and *Escherichia*), indicating a general circulation of these species in poultry production. Drinking water probably is not their preferred niche, but their presence in water is a consequence of contamination from other sources where these bacteria are among the most abundant.

Feed microbiota. Surprisingly, few data exist on feed microbiota despite its importance for chicken production. Feed may act as an important source of microbiota for the chickens, including pathogens like *Salmonella* (21,22,23). Volf *et al.* (5) detected mostly plant-associated (*Pantoea* and *Erwinia*) or soil- and environment-associated (*Curtobacterium*, *Pseudomonas*, *Stenotrophomonas*, and *Sphingomonas*) bacteria in the commercial feed of chickens. Another study from Haberecht *et al.* (1) ended with slightly different results, and in the feed, they detected bacteria common to litter (*Acinetobacter*, *Aerococcus*, *Brachybacterium*, *Brevibacterium*, *Comamonas*, *Corynebacterium*, *Dietzia*, *Facklamia*, *Jeotgalicoccus*, *Lactobacillus*, *Pseudomonas*, *Sphingobacterium*, and *Staphylococcus*) or fecal material (*Bifidobacterium*, *Lactobacillus*, *Blautia*, *Clostridium*, *Coprococcus*, *Oscillospira*, *Ruminococcus*, or *Turicibacter*). Plant microbiota can be considered as characteristic for the feed. The soil and environmental microbiota either may originate from the components used for feed production (1) or may be a consequence of secondary contamination at farms from the litter. However, the technological process of producing granulated feed is of such a high standard that freshly produced feed contains low levels of bacterial contamination. Moreover, it is not clear whether detected bacterial DNA in the feed corresponds to inactivated or viable bacteria. Bacterial culture from the feed resulted in growth of *Bacillus licheniformis*, *Clostridium symbiosus*, and *Lactobacillus inners*; i.e., different species from those detected by DNA sequencing (5). This finding indicates that plant and soil bacteria in the feed are probably no longer viable and sequencing signals originate from dead but not yet disintegrated cells. On the other hand, viable *B. licheniformis* and *C. symbiosus* likely survived the feed production process in the form of endospores.

Clean bedding microbiota. Similar to feed microbiota, not that much is known about the microbiota of fresh bedding. Different bedding material can be used in poultry production, including straw of various plant origins, saw dust, or even shredded paper (2). Bedding material has been reported to affect chicken performance and cecal microbiota composition. The effect was recorded during the first days of production and decreased with increasing time (2). Straw bedding may contain *Pantoea*, *Chryseobacterium*, *Citrobacter*, *Halomonas*, *Pedobacter*, *Pseudomonas*, *Sphingobacterium*, *Sphingomonas*, or *Xanthomonas* (24). This information indicates the presence of a mixture of plant (*Pantoea*) and environmental (all the remaining) bacteria. Our unpublished data show that clean bedding contains *Sphingomonas*, *Acinetobacter*, *Escherichia*, *Pedobacter*, *Flavobacterium*, *Pseudomonas*, *Chryseobacterium*, [*Ruminococcus*] *torques*, *Brevundimonas*, *Massilia*, *Stenotrophomonas*, *Rhizobium*, or *Sphingobacterium* as the most abundant genera. This list overlaps with published data (24) indicating that these bacteria are indeed characteristic straw bedding microbiota. Interestingly, when straw bedding was used in experimental studies, plant species of the straw were not mentioned. Although other factors may dominate over the straw plant species, attention should also be given to this point.

Litter microbiota. Unlike previous matrices, litter microbiota has been studied more frequently, although mostly in broilers; i.e., only for approximately 1 month of flock age. How litter microbiota develops further in reproductive flocks or egg layers is less clear. Most litter microbiota is represented by species that do not enter and multiply in the intestinal tract. Litter microbiota during the first month after chicken placement contains *E. coli* and *Lactobacilli* originating from the chicken intestinal tract. However, as the litter environment gradually develops, Firmicutes from orders Lactobacillales (*Aerococcus*, *Facklamia*, *Enterococcus*, and *Lactobacillus*) and Staphylococcales (*Staphylococcus*, *Jeotgalicoccus*, and *Salinicoccus*), Actinobacteria from orders Corynebacteriales (*Corynebacterium* or *Dietzia*) and Micrococcales (*Brevibacterium* or *Brachybacterium*), and Bacteroidetes from order Sphingobacteriales (*Sphingobacterium*) represent the most frequent litter microbiota members (2,3,4,17,25,26,27,28). Except for Lactobacilli and *E. coli*, the remaining genera do not belong among gut microbiota members. However, these genera are detected in the air dust, crop, skin, and respiratory tract microbiota (3,29,30,31) showing that they are in an interaction with the chicken host (32).

Skin microbiota. Skin microbiota is another example where not that much information is available for chickens. Although not directly representing skin microbiota of live chickens, chicken skin microbiota of broilers at slaughterhouses contained *E. coli*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Staphylococcus lentus* (29). Whole broiler carcass washes tested in another study contained *Gallibacterium anatis*; different Lactobacillli species; and low amounts of *Helicobacter canadensis*, *Phascolarctobacterium faecium*, *Bacteroides dorei*, and *Brevibacterium oceani* (4). Human skin microbiota, although depending on the skin site, is dominated by Gram-positive bacteria from genera *Staphylococcus*, *Propionibacterium* (currently *Cutibacterium*), and *Corynebacterium* (33,34). Lactobacilli were detected as members of human skin microbiota but did not belong among the most abundant and characteristic bacteria. Our unpublished data on chicken skin microbiota at the dorsal part of the chicken neck showed that ubiquitously distributed [*R.*] *torque* and *E. coli* were common in skin microbiota (Supplemental Table S4 and section "Bacteria tightly associated with poultry production" below). Additional skin microbiota members include *Blautia argi*, *C. disporicum*, *Cutibacterium acnes*, and Lactobacilli; e.g., *Lactobacillus johnsonii*, *L. aviarius*, *Lactobacillus gallinarum*, *Lactobacillus oris*, and *Lactobacillus salivarius*. Rather unexpectedly, *Megamonas hypermegale* that is common to chicken cecum was frequently found among skin microbiota as well. Since *M. hypermegale* is a strict anaerobe, its presence is likely due to contamination from fecal material.

Respiratory tract microbiota. Staphylococci, Streptococci, and Enterococci belong to regular bacterial species colonizing trachea (3,35). Tracheal Staphylococci are represented mostly by *S. epidermidis*; i.e., different from Staphylococci in the litter that belong to *Staphylococcus xylosus*, *Staphylococcus saprophyticus*, *Staphylococcus equorum*, and *Staphylococcus* (currently *Mammaliicoccus*)

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lentus. In addition, species dominant in other compartments can be detected in the trachea as well. They include *Brevibacterium*, *Brachybacterium*, or *Xanthomonas* (3,36,37) from the external environment or [*R.*] *torque* and *Blautia* (3,35) from fecal material. When skin and tracheal microbiota were compared, these two aerobic compartments we colonized by similar bacterial species including *B. argi*, *C. disporicum*, *C. acnes*, *Staphylococcus epidermidis*, and different *Lactobacillus* sp.

The lower respiratory tract is lowly populated. Wang *et al.* (38) reported similar a microbiota composition in bronchoalveolar lavage as in trachea, i.e., *Lactobacillus*, *Staphylococcus*, and *Corynebacterium* accompanied by minority bacteria *Ruminococcus*, *Blautia*, *Coprococcus*, *Butyricicoccus*, *Eubacterium*, *Dorea*, *E. coli*, and *Phascolarctobacterium*, likely from inhaled remains of fecal material. On the other hand, Shabbir *et al.* (39) reported lung microbiota as quite different from trachea and containing *Avibacterium*, *Pseudomonas*, or *Bordetella*. Lung microbiota usually contains *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *C. disporicum*, *E. coli*, and *C. acnes*, of which their origin can be seen in other aerobic compartments like the skin. The origin of another group of lung microbiota like *Blautia*, [*R.*] *torques*, *Butyricicoccus*, *Faecalibacterium*, or *Bacteroides* is likely in the intestinal tract spreading to lungs via environment and airways because studies performed with young chickens report Firmicutes as dominating in the lung (40,41), whereas adult hen lung microbiota contains an increased amount of Bacteroidetes (42), corresponding with a low representation of Bacteroidetes in young chickens but their increased presence in adult hen gut microbiota (15). *Pseudomonas*, *Bordetella*, and *Serratia* therefore represent genera characteristic for the lower respiratory tract, which are not commonly reported for other compartments.

Crop microbiota. The crop is a specific organ of aves. Besides feed moistening and initial fermentation, the crop serves as a first barrier against pathogens entry. The crop environment is characterized by the presence of lactic acid and a pH between 4.8 and 5.0 (43,44). This environment is affected by microbial colonization of the crop that is dominated by *Lactobacillus* species. The dominance of Lactobacilli is extreme because multiple papers report Lactobacilli abundance in the crop between 95% and 99% (18,43,44,45,46,47,48). Interestingly, although there are reports on the poor colonization ability of Lactobacilli in the cecum (49), oral administration of Lactobacilli results in efficient crop colonization (46). Of the minority species in the crop, *G. anatis* can be considered as capable of colonizing the crop (7,45), whereas the presence of *Xanthomonas*, *Corynebacterium*, *Staphylococcus*, *Acinetobacter*, *Aeromonas*, or *Comamonas* is likely a mere consequence of their ingestion from the environment (18,44,46,48).

Small intestine microbiota. Due to the specific conditions in the small intestine, the ileal microbiota is less diverse than the cecal or litter microbiota. Rapid digesta transition and the presence of digestive enzymes and bile salts does not allow many bacterial species to survive and colonize. This environment is also the reason why absolute bacterial counts per gram of digesta are approximately 100× lower in the small intestine than that in the cecum. Despite minor differences, different parts of the small intestine, i.e., duodenum, jejunum, and ileum, are colonized by microbiota of a similar composition (50,51,52). In the proximal part of the small intestine, Cyanobacteria are recorded by DNA sequencing, but this is caused by the presence of plant chloroplast DNA from yet-not-digested feed (53). On the other hand, microbial diversity sometimes increases in the distal part of the small intestine due to mixing of ileal digesta with digesta from the cecum (50). Small intestine microbiota is dominated by different *Lactobacillus* species, *Romboutsia*, and *Turicibacter*. Lactobacilli commonly form around 80% of the total small intestine microbiota (3,43,44,47,54) and together with the latter two genera over 90% (1,10). Common species include *L. johnsonii*, *Lactobacillus reuteri*, *L. gallinarum*, *L. salivarius*, and *L. aviarius* (45), and consequently, the most common organic acid in the small intestine is lactic acid (44). As Lactobacilli are quite widespread in the environment, colonization of the small intestine is quite rapid and can be accomplished within the first week of life (10).

Microbiota in the cecum. The environment in the cecum is strictly anaerobic, with a constant temperature and continuous nutrient supply. This environment makes the cecum different from all other niches described in this review, and this is also a reason why cecal microbiota differs from the rest (Fig. 2). The cecum is a densely populated part of the chicken intestinal tract with 10^{10} CFU/g of digesta and up to 1000 different bacterial species. The most characteristic organic acid for the cecum is acetate followed by propionate and butyrate (44). When characterizing cecal microbiota in chickens, one has to be very careful about chicken age. This, apparently in disagreement, does not mean that the composition of the cecal microbiota is strictly dependent on chicken age, as chicks can be colonized by the adult type of microbiota from the first hours of their life (8,40,55,56). However, because nearly all chickens are hatched in hatcheries, i.e., without contact with adult birds, which would act as sources of normal gut flora, the development of cecal microbiota in chicks from hatcheries takes some time. It is impossible to define the time required for the establishment of final cecal microbiota because the colonization of chicks from hatcheries is a mere matter of coincidences (16). The higher the zoohygienic standards, the longer the development will last. But even in this case, the previous statement is based only on probability, which does not exclude the rapid colonization of chicks from hatcheries if an appropriate source is available. The observed gradual colonization is only a consequence of hatching in the absence of parents. Moreover, due to the absence of parents, cecal microbiota in the chicks during the first days of life does not represent true chicken cecal microbiota, as this is the microbiota of environmental origin capable of multiplication in the chicken cecum (8). Collectively, if a realistic view on the structure of the chicken microbiota is the aim, it is important to analyze samples from adult hens. This, of course, does not exclude the necessity of understanding the structure of the cecal microbiota in chickens of broiler age.

Cecal microbiota of chicks of up to 5 weeks of age usually consists of *E. coli* (Proteobacteria) and Gram-positive Firmicutes. Within Firmicutes, representatives of families *Lachnospiraceae*, *Ruminococcaceae*, *Oscillospiraceae*, *Erysipelotrichaceae*, and *Lactobacillaceae* dominate (18,43,44,46,48,54). The reason for this is that these microbiota members are either spore-forming bacteria (*Lachnospiraceae*, *Ruminococcaceae*, *Oscillospiraceae*, and *Erysipelotrichaceae*) or aerotolerant (*Lactobacillaceae*) (57). Such bacteria survive in the form of spores in the environment from which they continuously enter the chicken intestinal tract (58). *Bacteroidaceae*, *Rikenellaceae*, and *Porphyromonadaceae* from phylum Bacteroidetes may appear in gut microbiota of young chickens as well (1,3,18,46,47), but the likelihood of their presence in the cecal microbiota during first 10 days of life is rather low. In agreement with previous statements, the more contained conditions and better zoohygienic rules at animal

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houses, the longer, on average, it takes for Bacteroidetes to appear in the cecum.

Cecal microbiota of adult hens consists of approximately a similar representation of Firmicutes and Bacteroidetes (8,15). In Firmicutes, representatives of the order Selenomonadales (genera *Megamonas*, *Megasphaera*, *Dialister*, or *Phascolarctobacterium*) appear with the same developmental pattern as Bacteroidetes (15). Another phylum commonly recorded in the cecum of adult hens is represented by Actinobacteria with genera *Bifidobacterium*, *Olsenella*, or *Collinsella*. Proteobacteria are also present in the cecum of adult hens, although *E. coli* represents a minority species and is replaced by *Sutterella*, *Parasutterella*, *Succinatimonas*, or *Anaerobiospirillum*. Other phyla appear only occasionally. Fusobacteria sometimes overgrow and are usually an indicator of incorrect cecal microbiota function (10). However, whether this is a cause or consequence is not known. Elusimicrobia, Synergistetes, Spirochaeta, and Defferribacteres are also present in the cecal microbiota, although if cecal digesta is collected, the abundance of these phyla rarely exceeds 0.1%. Our current unpublished data show that this could be caused by misunderstanding the spatial distribution of at least some of these phyla. Representatives of Deferribacteres and Spirochaeta are localised to mucosal surfaces, and if they are collected from adult hens, these bacteria may form between 5% and 10% of the cecal mucosal microbiota (unpublished observations and 59).

Air and air dust microbiota. Air and air dust microbiota in poultry houses are similar to litter and fecal microbiota (54,60,61), although there are also reports concluding no similarity between fecal and air microbiota (62). However, the latter study reported only cluster analyses that does not exclude the presence of the same species both in the fecal and air dust microbiota but at different abundance. Air microbiota in poultry houses is dominated by Gram-positive bacteria with different Staphylococci reported the most frequently (31,54,60). Staphylococci are present in a range between 10^6 and 10^7 CFU/m^3, thus representing a health risk for human personnel (30). Air dust may also contain bacteria of intestinal origin like *Bacteroides*, *Phascolarctobacterium*, *Romboutsia*, *Blautia*, or *Faecalibacterium* (60,63) or those from the litter like *Acinetobacter*, *Corynebacterium*, *Brachybacterium*, *Aerococcus*, *Brevibacterium*, *Jeotgallicoccus*, *Weissella*, *Facklamia*, *Oceanobacillus*, or *Yaniella* (54,60). Due to the absence of nutrients in the air, air bacteria do not proliferate but air with dust particles with adsorbed bacteria act as a vehicle interconnecting nearly all niches within chicken production, i.e., intestinal and litter microbiota with water, skin, and respiratory tract microbiota. Interestingly, air microbiota of litter origin (*Brachybacterium*, *Aerococcus*, *Brevibacterium*, *Jeotgallicoccus*, *Weissella*, *Facklamia*, *Oceanobacillus*, or *Yaniella*) do not enter and persist in the respiratory tract, whereas microbiota (or at least their DNA) of gut origin (*Bacteroides*, *Phascolarctobacterium*, *Romboutsia*, *Blautia*, or *Faecalibacterium)* can be detected in the respiratory tract, including the lung.

Bacteria tightly associated with poultry production. At least some of the microbiota members within poultry production can be found in different matrices. Litter microbiota contain microbiota from the small and large intestine (3,25,32,61,64), and crop microbiota may contain air dust, litter, and cecal microbiota members (48). The intact eggshells microbiota is a mixture of cecal and environmental microbiota (5). Microbiota from feed collected at farms can be contaminated by low amounts of litter microbiota. Overlaps and interactions between microbiota members from different ecological compartments have also been confirmed indirectly by the presence of the same antibiotic resistance genes circulating in intestinal and air dust microbiota (62).

Because the microbiota composition in our samples did not differ extensively from already published data, finally, we used our dataset (Supplemental Table S2 and S3) for the identification of the most common bacteria along poultry production. The following ideas might be taken critically because they are based on an analysis of a limited number of samples, but they may be also taken as a future challenge and hypothesis worthy of future investigation. *Escherichia coli* was the most widespread bacterium associated with chicken production because it was detected in 144 out of the 174 samples included. *Escherichia coli* dominated in aerobic compartments and was not that competitive in the intestinal samples (Fig. 4). [*Ruminococcus*] *torques* was the second most common bacterium in chickens present in 130 samples. The 16S ribosomal RNA (rRNA) sequence of chicken [*R.*] *torques* was only 97.76% similar to the [*R.*] *torques* entry in GenBank, so this isolate may be reclassified as a novel species in the future. *Lactobacillus gallinarum* was another species present in 131 samples out of 174 tested. Other Lactobacilli species were also quite common along the chicken production, and they included *L. salivarius* present in 118 samples, *L. reuteri* ($n = 116$), and *L. johnsonii* ($n = 111$). Lactobacilli dominated in the samples of skin, trachea, crop, and ileal microbiota, but due to their aerotolerance, they were also able to survive in different types of environmental samples. A trend for adaptation to different compartments within the chicken has been recorded. *Lactobacillus salivarius* preferentially colonized the crop, whereas *L. gallinarum* preferentially localized to the ileum, although this finding will have to be confirmed in future studies. *Romboutsia timonensis* (family Peptostreptococcaceae) was detected in 119 and *C. disporicum* in 100 samples of 174 samples tested. *Romboutsia timonensis* is common to the small intestine, and because the passage of digesta through the small intestine is quite rapid, a lot of fecal material enriched for *R. timonensis* is released into the environment. Consequently, eggshells and skin were commonly contaminated, whereas fresh clean bedding was commonly free of this bacterium (Fig. 4). *Clostridium disporicum* was another broadly distributed but rather unexpected bacterium in chicken production. *Clostridium disporicum* was common in hatcheries where it formed around 10% of microbiota in eggshell waste, and it was also detected among skin microbiota in increased representation (Fig. 4). Despite the low abundance, it was also present in other niches within the whole chicken production. Other bacterial species common to chicken production were those colonizing the chicken cecum. These bacteria were recorded in 60–80 samples out of 174 tested. The source of all these bacteria was the cecum, and these bacteria could be detected on intact eggshells and in decreasing abundance in the samples of skin, trachea, crop, and ileum. Because these bacteria are continuously voided from the cecum, they could also be detected among litter microbiota.

Concluding remarks and future challenges. In this review, we have shown that except for the trachea, skin, and crop, top microbiota members in the remaining types of samples were different among themselves. Major clean bedding microbiota or feed or drinking water microbiota members do not persist in the litter or intestinal tract, major litter or eggshell waste microbiota members do not colonize the intestinal tract, and cecal anaerobes do not multiply in the litter. However, these results do not exclude cross contamination. Major intestinal tract microbiota members are found in the litter, and with decreasing abundance also in the skin,

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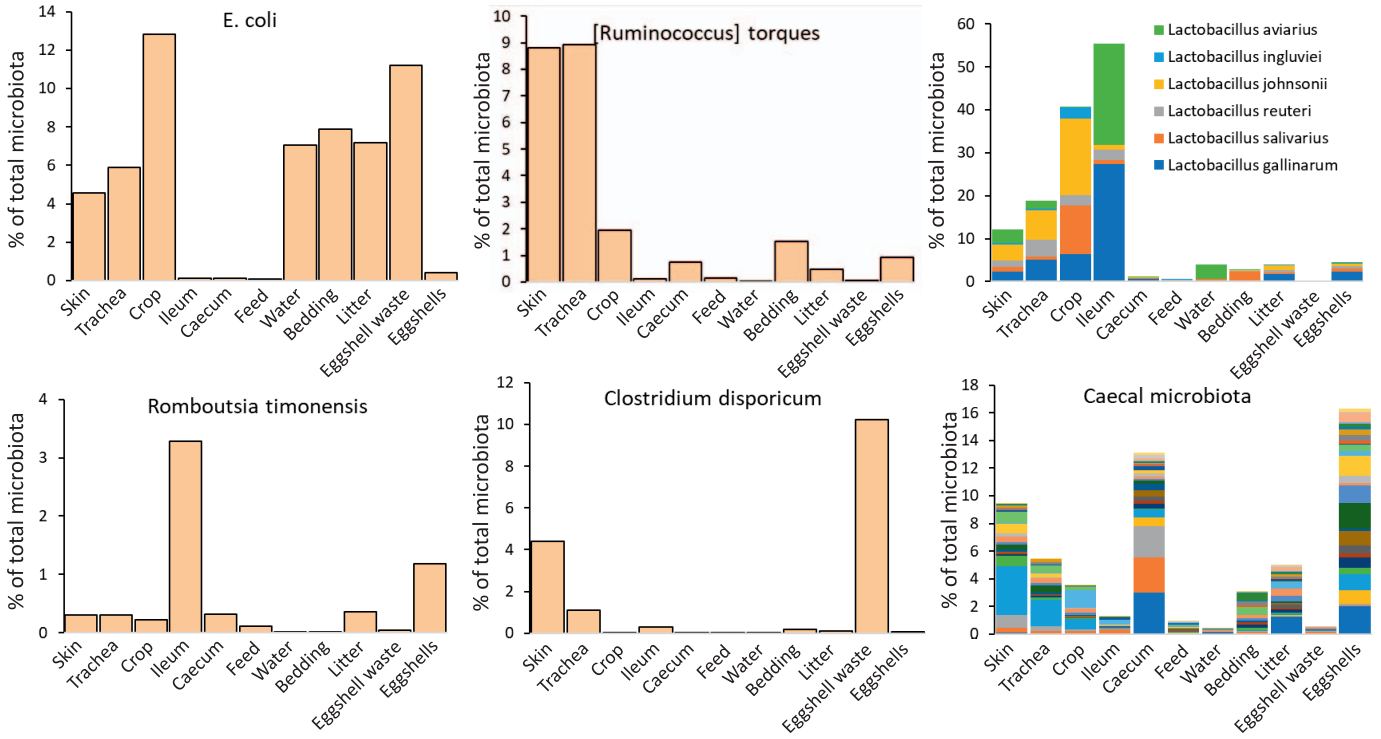

Fig. 4. The most abundant bacterial species in chicken production. *Escherichia coli* is a universal bacterium capable of replication in different ecological niches. However, its presence in gut microbiota is minimal in comparison to its colonization of aerobic environments. On the other hand, Lactobacilli require environments associated with animals except for the distal part of the intestinal tract. *Romboutsia timonensis* spread to the rest of the environments from the ileum and a similar result can be concluded for different cecal microbiota members. *Clostridium disporicum* was common in the eggshell waste and likely from this source contaminated and colonized chicken skin and trachea from where it spread to other compartments. Please, see Supplemental Table S4 for the identification of cecal microbiota OTUs.

trachea, crop, and ileum (Fig. 4). Top litter microbiota members are present in the air dust, skin, crop, or trachea as minority members. Because even minority members of the litter or air dust microbiota can be a source of infection for the chicken intestinal and respiratory tract, a caution should be paid to microbial composition in chicken environment.

There are many studies describing experiments with the modification of gut microbiota composition, either via administration of probiotics or by modification of feed composition (27,40,43,46,52,56). Much less attention is given to the active manipulation of the respiratory tract or skin microbiota. These compartments are less densely populated, but this does not necessarily mean that their active colonization with beneficial microbes is not important. A broad distribution of spore-forming [*R.*] *torque* and *C. disporicum* may explain the efficacy of *Bacillus* spores in stimulating the chicken immune system (65,66) by mimicking the natural inoculation of chickens by spores of [*R.*] *torque* and *C. disporicum*. But perhaps even more underdeveloped are the abiotic compartments along chicken production. Will it be possible to identify bacterial species with an antagonistic relationship in these environments? Although it might be technologically and economically difficult to modify bedding and litter microbiota due to too-high volumes of the material to be inoculated and treated, there are places within poultry production that are much more concentrated and localized. Following such a deep understanding, it might be possible to select bacterial species for spraying eggs after cleaning and disinfection, to prevent the multiplication of undesired microbiota on the eggshells during embryonic development. Similarly, it might be possible to spray eggs with competitive microbiota not intended for chicken colonization but for the suppression of *E. coli* or *Acinetobacter* multiplication in eggshell waste from hatcheries. Chicken production in these critical points is quite concentrated so that the volume of bacterial cocktails used for biological cleaning need not be that high. If such interactions are developed in detail, it might be possible to reduce the volume of cleaning chemistry and replace it with biological approaches that are friendlier to the environment. To reach this position, an initial, detailed characterization of microbiota in different compartments followed by an understanding their mutual interactions within or across different compartments is indeed needed. Probiotics for gut colonization can be combined with probiotics for the respiratory tract, and it can be combined with microbiological products intended for environment colonization. Although individually such interventions may have a limited effect, their mutual combination may lead to positive synergistic interactions resulting in sustainable and environmentally friendly production of poultry.

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ACKNOWLEDGMENTS

We thank Peter Eggenhuizen for language corrections. This work has been supported by projects RVO0518 and QK22020066 of the Czech Ministry of Agriculture.

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