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# Contact with adult hens affects the composition of skin and respiratory tract microbiota in newly hatched chicks

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**ABSTRACT** Chickens in commercial production are hatched in hatcheries without any contact with their parents and colonization of their skin and respiratory tract is therefore dependent on environmental sources only. However, since chickens evolved to be hatched in nests, in this study we evaluated the importance of contact between hens and chicks for the development of chicken skin and tracheal microbiota. Sequencing of PCR amplified V3/V4 variable regions of the 16S rRNA gene showed that contact with adult hens decreased the abundance of *E. coli, Proteus mirabilis* and *Clostridium perfringens* both in skin and the trachea, and *Acinetobacter johnsonii* and *Cutibacterium acnes* in skin microbiota only. These species were replaced by *Lactobacillus gallinarum, Lactobacillus aviarius, Limosilactobacillus reuteri*, and *Streptococcus pasterianus* in the skin and tracheal microbiota of contact chicks. *Lactobacilli* can be therefore investigated for their probiotic effect in respiratory tract in the future. Skin and respiratory microbiota of contact chickens was also enriched for *Phascolarctobacterium, Succinatimonas, Flavonifractor, Blautia*, and [*Ruminococcus*] *torque* though, since these are strict anaerobes from the intestinal tract, it is likely that only DNA from nonviable cells was detected for these taxa.

**Key words:** chicken, skin, trachea, caecum, respiratory tract microbiota

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## INTRODUCTION

Chickens in commercial production are hatched in hatcheries without any contact with their parents. Colonization of their skin, respiratory, and intestinal tract by bacterial communities is therefore dependent on environmental sources only. We have earlier shown that contact with adult hens is vital for the development of cecal microbiota in newly hatched chicks (Varmuzova et al., 2016; Kubasova et al., 2019). While the establishment of gut microbiota characteristic of adult chickens may take months in chickens from hatcheries (Videnska et al., 2014), the establishment of adult-type cecal microbiota in chicks in contact with adult hens requires less than 1 wk of life (Kubasova et al., 2019; Marcolla et al., 2023). Colonization of the skin and respiratory tract can be also affected by contact with adult hens. Beneficial skin microbiota may protect chicks against infections after minor injuries and respiratory tract microbiota may increase resistance to respiratory disorders caused by opportunistic pathogens like *Staphylococcus* or *Acinetobacter* present in the air of poultry farms (Oppliger et al., 2008; Liu et al., 2019; Bindari et al., 2021).

The respiratory tract of broiler chickens is colonized by *Lactobacillus, Enterococcus, Streptococcus, Escherichia coli, Acinetobacter, Pseudomonas* and *Corynebacterium* (Johnson et al., 2018; Ngunjiri et al., 2019; Abundo et al., 2021; Zhou et al., 2021). In addition, [*Ruminococcus*] *torques, Blautia, Faecalibacterium, Butyricicoccus, Romboutsia*, and *Phascolarctobacterium*, all characteristic of gut microbiota (Stanley et al., 2013; Videnska et al., 2014; Ranjitkar et al., 2016), can be found in the chicken respiratory tract (Wang et al., 2020a; Abundo et al., 2021; Zhou et al., 2021). *Weissella, Xanthomonas, Brachybacterium*, and *Brevibacterium* were also reported as part of respiratory tract microbiota (Sohail et al., 2015; Johnson et al., 2018). These bacteria originate in the litter (Johnson et al., 2018) and these species are usually missing in the respiratory tract microbiota in short-term experiments (Wang et al., 2020a; Abundo et al., 2021; Zhou et al., 2021) that do not allow complex litter formation (Kubasova et al., 2022). Microbiota of the respiratory tract in adult egg layers, in addition to all the above-mentioned species, may also contain *Avibacterium, Gallibacterium, Mycoplasma*, and representatives of phylum Bacteroidetes



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(genera *Bacteroides, Odoribacter* or *Alistipes*) (Glendinning et al., 2017; Ngunjiri et al., 2019; Taylor et al., 2020; Van Goor et al., 2020; Wang et al., 2020b). Bacteroidetes originate from the intestinal tract and their late appearance in the respiratory tract can be associated with their late appearance in the caecum (Videnska et al., 2014). However, the composition of respiratory tract microbiota has been always characterized in chickens from hatcheries.

Skin microbiota of chickens has been studied less frequently than respiratory tract microbiota, mostly in broiler carcasses due to the risk of pathogen transmission into the human food chain (Hinton and Ingram, 2000; Oakley et al., 2013; Meng et al., 2019; Zhang et al., 2020). These studies reported the presence of *E. coli, Enterococcus, Lactobacillus, Gallibacterium, Pseudomonas, Acinetobacter*, and *Campylobacter*. Reports on human skin microbiota mentioned the presence of species belonging to genera *Staphylococcus, Cutibacterium* (previously *Propionibacterium*), and *Corynebacterium* (Grice and Segre, 2011; Flowers and Grice, 2020).

Due to extensive commercial production and hatching in hatcheries, it is commonly forgotten that chickens evolved to be hatched in nests, in contact with their parents, which could act as a source of chicken-adapted microbiota. Nothing is known about the transfer of skin and respiratory tract microbiota between adult hens and chicks, although it is known that such contact considerably affects the development of cecal microbiota (Kubasova et al., 2019) and increases resistance to *Salmonella* infection (Rantala and Nurmi, 1973; Varmuzova et al., 2016).

In this study, we therefore compared skin and trachea microbiota of 1-wk-old chicks kept with or without adult hen contact. Considerable differences in microbiota composition in the trachea and skin of control and contact chicks were recorded, which can be investigated for their probiotic effect in the respiratory tract or skin microbiota of newly hatched chicks in commercial production.

## MATERIALS AND METHODS

### Ethical Statement

The handling of animals in the study was performed in accordance with current Czech legislation (Animal Protection and Welfare Act No. 246/1992 Coll. of the Government of the Czech Republic). The specific experiments were approved by the Ethics Committee of the Veterinary Research Institute followed by the Committee for Animal Welfare of the Ministry of Agriculture of the Czech Republic (permit number MZe 2186).

### Experimental Chickens and Hens

In all experiments, newly hatched male ISA Brown chicks were obtained from a local commercial hatchery on the day of hatching. Contact hens acting as a natural source of microbiota were obtained from a local commercial egg-laying hen farm. Chicks were reared in plastic boxes with perforated floor, with free access to water and standard starter feed.

### Microbiota Transfer by Contact

Ten newly hatched chicks were divided into 2 groups. Five chicks in the experimental group were reared in a cage together with a 45-wk-old hen. Chicks in the control group were kept under the same conditions but without any contact with an adult hen. Seven days later, all chicks and contact donor hen were humanely sacrificed, and approximately 20 $mm^2$ of skin from the dorsal part of the neck, 20 $mm^2$ of trachea, and 0.5 g of cecal content were collected. Collected samples were frozen at −20°C within 15 min after dissection and total DNA for microbiota characterization was purified within 1 mo after sample collection. This experiment was repeated 3 times at completely independent occasions in exactly the same way.

### Microbiota Characterization by 16S rRNA Sequencing

The samples were thawed, homogenized in a MagNA-Lyzer (Roche, Rotkreuz, Switzerand) and the DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The DNA concentration was determined spectrophotometrically and samples diluted to 5 ng/mL were used as a template DNA in PCR (Polymerase Chain Reaction) as described previously (Kubasova et al., 2022). The resulting PCR products were purified using AMPure beads. In the next step, the PCR product concentration was determined with a spectrophotometer before the DNA was diluted to 100 ng/$\mu$L and labeled with indices from Nextera XT Index Kit (Illumina, San Diego, CA). Prior to sequencing, the concentration of indexed samples was determined using a KAPA Library Quantification Complete kit (Kapa Biosystems, Wilmington, MA). All indexed samples were diluted to 4 ng/$\mu$L and 20 pM phiX DNA was added to a final concentration of 5% (v/v). Sequencing was performed using MiSeq Reagent Kit v3 (600 cycles) and MiSeq apparatus (Illumina, San Diego, CA).

Postsequencing analysis was performed with QIIME 2 (Bolyen et al., 2019). Raw sequence data were demultiplexed, quality filtered and sequencing primers were trimmed using in-house scripts utilizing Je (Girardot et al., 2016) and fastp (Chen et al., 2018). The resulting sequences were denoised with DADA2 (Callahan et al., 2016). Taxonomy was assigned using the q2−feature−classifier (Bokulich et al., 2018) and using the classify−sklearn naïve Bayes taxonomy classifier against the Silva 138 database (Quast et al., 2013). Taxonomic identification at the lowest level (amplicon sequence variants [**ASV**]) was assigned using 99.9% similarity to entries in the Silva database.

### Statistical Analysis

Chao1, Shannon index and principle coordinate analysis (**PCoA**) using Bray−Curtiss matrix distances were calculated by Qiime 2. PERMANOVA and Mann−

Whitney test (R-project, package vegan, Adonis function followed by pairwise comparisons) were used to determine taxa significantly differing among individual groups of samples.

## RESULTS

### Sequencing Data

Since 1 chick in contact with an adult hen died, samples of 14 contact chicks, 15 control chicks, and 3 donor hens were analyzed. As 3 tissues (trachea, skin, caecum) were processed from each chicken or hen, 96 samples characterized by a total of 2,712,319 reads were available for downstream analyses. This represented an average coverage of 28,253 reads per sample, with a maximal sample coverage 98,042 reads and a minimal sample coverage 2,579 reads. Chao1 species estimate showed that there were more species colonizing the skin and trachea than the caecum. Contact with an adult hen resulted in a significantly increased number of species in the caecum but not on the skin or in the trachea (Figure 1A). Species diversity was higher in skin or tracheal microbiota compared to cecal microbiota. Microbial diversity in all tested compartments was always higher in the contact group than in control chickens (Figure 1B).

Although the main aim was to identify differences in tracheal and skin microbiota in control and contact chicks, first we briefly checked for cecal microbiota. Tracheal and skin microbiota was then analyzed in 3 steps, 1) the most common microbiota members in the trachea and skin, 2) microbiota members differently abundant in the trachea and skin, and 3) microbiota members differently abundant in the skin and trachea of control and contact chicks.

### Cecal Microbiota in Control and Contact Chicks

Cecal microbiota was analyzed only for control purposes, to confirm effective contact between hens and chicks. In agreement with previous reports (Varmuzova et al., 2016; Kubasova et al., 2019). Proteobacteria decreased and Bacteroidetes increased in the cecal microbiota of contact chicks in comparison to control chicks (Figure 2A). This confirmed efficient contact between hens and chicks and allowed to focus on skin and trachea microbiota in the rest of this report.

### The Most Common Tracheal and Skin Microbiota Members

Major families in skin and tracheal microbiota in control chicks included Enterobacteriaceae and Oxalobacteraceae from phylum Proteobacteria, and Lactobacillaceae, Lachnospiraceae, Clostridiaceae, and Selenomonadaceae belonging to phylum Firmicutes. These families formed approximately 65% of all skin and trachea microbiota in control chicks (Figure 2A).

When looking at the most widespread ASVs in skin samples, [*Ruminococcus*] *torques, E. coli, Blautia argi*, [*Ruminococcus*] faecis, *Clostridium disporicum, Clostridium perfringens, Cutibacterium acnes, Sphingomonas hankookensis, Lactobacillus gallinarum, Flavonifractor plautii, Proteus mirabilis, Novosphingobium subterraneum, Thomasclavelia ramose*, and *Acinetobacter johnsonii* were present in 14 or 15 samples out of 15 skin samples of control chicks (Figure 2B).

Amplicon sequence variants present in all or absent in only single tracheal samples of control chicks included *B. argi*, [*Ruminococcus*] *torques, E. coli*, [*Ruminococcus*] *faecis, L. reuteri, Romboutsia timonensis, L. gallinarum, C. acnes, F. plautii, C. perfringens, Corynebacterium sanguinis, Corynebacterium tuberculostearicum*, and *Anaerococcus nagyae* (Figure 2C).

Major families in skin and tracheal microbiota in chicks raised in contact with an adult hen included Oxalobacteraceae (phylum Proteobacteria), Lactobacillaceae, Lachnospiraceae, Clostridiaceae, Selenomonadaceae, and Ruminococcaceae (all phylum Firmicutes), and Bacteroidaceae and Rikenellaceae (both from phylum Bacteroidetes). These families

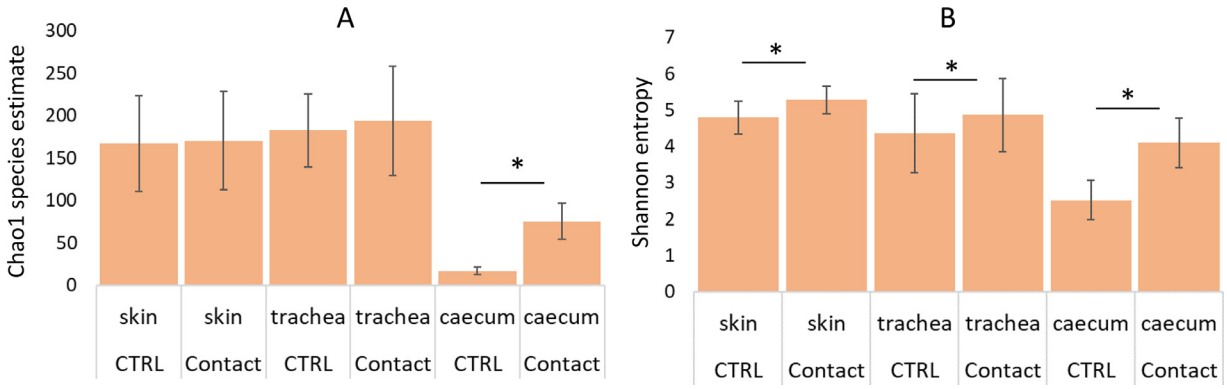


**Figure 1.** Microbial diversity in skin, tracheal and cecal microbiota. Skin and trachea were colonized by higher number of species than the caecum. Contact with adult hen significantly increased number of species in the caecum (Panel A). Bacterial diversity in the skin and trachea determined by Shannon entropy was higher than in the caecum. The diversity increased by the contact with adult hen in all compartments, t test, $P < 0.05$ (Panel B).

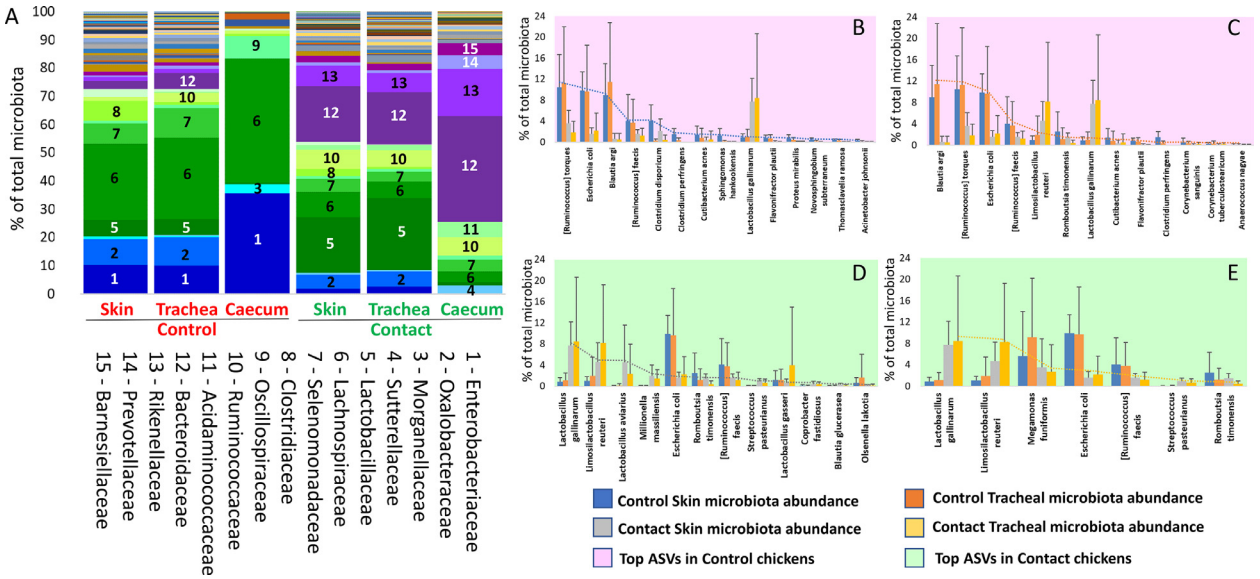


**Figure 2.** The most widely distributed taxa in skin or trachea of 1-wk-old chicks. Panel A, microbiota composition at family level. Shades of blue—families belonging to Proteobacteria, shades of green—families belonging to Firmicutes, shades of magenta—families belonging to Bacteroidetes. Panel B, the most widespread ASVs in skin samples of control chickens. Panel C, the most widespread ASVs in tracheal samples of control chickens. Panel D, the most widespread ASVs in skin samples of contact chickens. Panel E, the most widespread ASVs in tracheal samples of contact chickens. Dotted lines in panels B–E highlight descending arrangement of ASVs for each type of sample. Abbreviation: ASVs: amplicon sequence variants.

formed approximately 75% of all skin and tracheal microbiota in contact chicks (Figure 2A).

Amplicon sequence variants present in all or absent in only a single sample out of 14 skin samples of contact chicks included *L. gallinarum, L. reuteri, Lactobacillus aviarius, Lactobacillus gasseri, Millionella massiliensis, E. coli, R. timonensis*, [*Ruminococcus*] *faecis, Streptococcus pasteurianus, Coprobacter fastidiosus, Blautia glucerasea*, and *Olsenella lakotia* (Figure 2D).

Finally, *L. gallinarum, L. reuteri, Megamonas funiformis, E. coli*, [*Ruminococcus*] *faecis, S. pasteurianus*, and *R. timonensis* were present in all or absent in only a single tracheal sample of contact chicks (Figure 2E).

While Oxalobacteraceae belonged among common families in the skin and tracheal microbiota, not a single representative from this family has been mentioned above. A major representative of this family, *Janthinobacterium violaceinigrum*, was present in 12 skin samples of both control and contact chicks, in 12 tracheal samples of control chicks, and in 11 tracheal samples of contact chicks and it therefore did not pass the defined threshold in all groups by a single count. However, when present, *Janthinobacterium violaceinigrum* could form over 10% of skin or tracheal microbiota.

## Differently Abundant Skin and Trachea Microbiota Members

At the family level, there were 5 families (*Clostridiaceae, Enterococcaceae, Sphingomonadaceae, Staphylococcaceae*, and *Moraxellaceae*) each forming more than 0.5% of total microbiota that were more abundant in skin than in tracheal microbiota in control chickens. Not a single family with representation higher than 0.5% of total microbiota was more abundant in tracheal microbiota than in skin microbiota of control chickens (Figure 3A).

Four families, each forming more than 0.5% of the total microbiota, were differently abundant in skin and tracheal microbiota in contact chickens. Three of them (*Lachnospiraceae, Clostridiaceae*, and *Peptostreptococcaceae*) were more abundant in skin, and one of them (*Pasteurellaceae*) in tracheal microbiota (Figure 3B).

There were 119 ASVs, each forming more than 0.05% of the total skin microbiota in control chickens. Of these, 9 were significantly more abundant in skin than in tracheal microbiota (*C. disporicum, C. perfringens, Enterococcus faecalis, S. hankookensis, N. subterraneum, Stenotrophomonas geniculata, A. johnsonii, Zhenhengia yiwuensis*, and *Megamonas hypermegale*) (Figure 3C).

Out of 135 ASVs forming more than 0.05% of tracheal microbiota in control chickens, 7 of them (*Lactobacillus iners, Negativibacillus massiliensis, Succinatimonas hippei, Thomasclavelia spiroformis, Sphingobacterium cibi, Phascolarctobacterium faecium*, and *Rhodoligotrophos jinshengii*) were significantly more abundant in tracheal than in skin microbiota (Figure 3C).

Out of 161 ASVs forming more than 0.05% of the total skin microbiota in contact chickens, 8 were significantly more abundant in the skin than tracheal microbiota (*Lactobacillus aviarius, C. disporicum*, [*Ruminococcus*] *faecis, R. timonensis, B. glucerasea, Turicibacter bilis, Lachnoclostridium edouardi*, and *Staphylococcus equorum*) (Figure 3D). Not a single ASV out of the 170 ASVs forming more than 0.05% of total tracheal microbiota in contact chickens was more abundant in tracheal than in skin microbiota.

A rather low number of major ASVs differently abundant in skin and tracheal microbiota indicated that these compartments were colonized by similar consortia

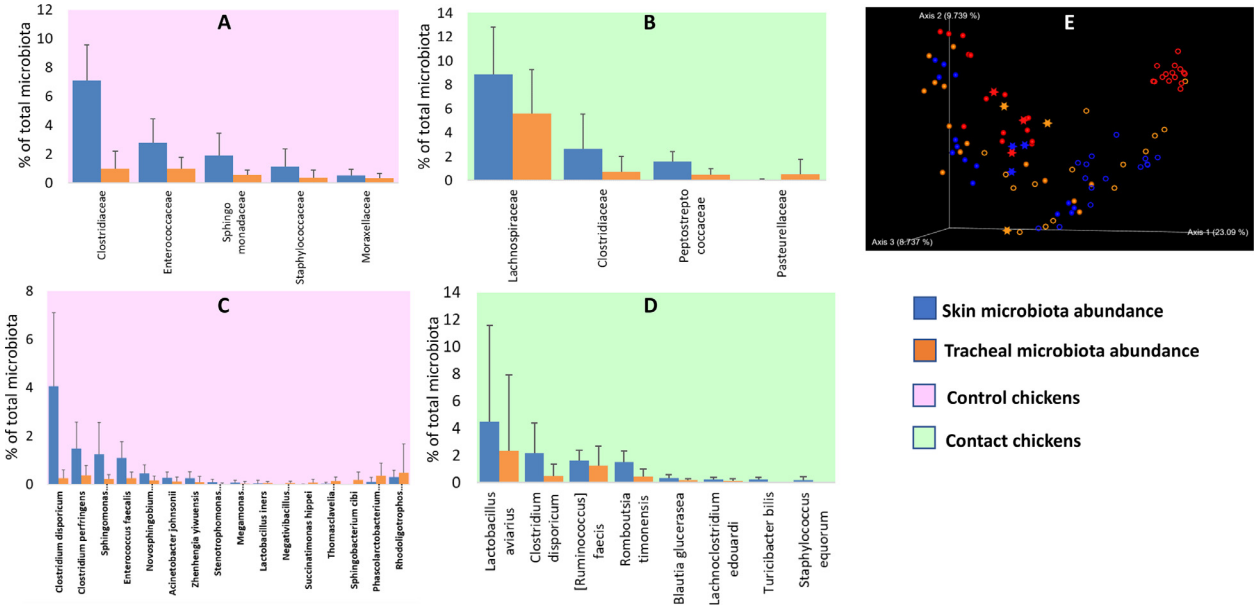


**Figure 3.** Differently abundant families and ASVs in skin or tracheal microbiota. Panel A, bacterial families differently abundant in skin and trachea of control chickens, panel B, bacterial families differently abundant in skin and trachea of contact chickens, panel C, ASVs differently abundant in skin and trachea of control chickens, panel D, ASVs differently abundant in skin and trachea of contact chickens. Panel E, PCoA using Bray Curtiss matrix distances. Red—caecum, blue—skin, orange—trachea, stars—donor hens, opened symbols—control chicks, filled circles—contact chicks. All taxa showed in panels A–D were differently abundant in skin or trachea microbiota by Mann–Whitney test ($P < 0.05$). Abbreviation: ASVs: amplicon sequence variants.

of microbiota. This was confirmed also by PCoA analysis in which skin and tracheal samples commonly overlapped (Figure 3E).

### *Differently Abundant Taxa in Skin and Tracheal Samples From Control and Contact Chickens*

Contact with an adult hen significantly affected the abundance of 162 and 144 ASVs in the skin and tracheal microbiota, respectively. Of these, 112 skin microbiota members were more abundant in contact chickens and 50 in control chickens. Similarly, 104 tracheal ASVs were more abundant in contact chickens and 40 in control chickens (Supplementary file 1).

We also tested an alternative view, similar to that used for the identification of the most characteristic taxa for skin and tracheal microbiota. First, ASVs that were present in 14 or 15 samples out of 15 skin or tracheal samples from control chickens, and 13 or 14 samples out of 14 skin or tracheal samples from contact chickens were selected and their differential abundance in control and contact chickens was evaluated in the next step. Twelve out of 20 ASVs were significantly more abundant in skin microbiota of control than in contact chickens and, vice versa, 7 ASVs were more abundant in skin samples from contact than from control chickens (Figure 4). Ten ASVs were more abundant in the trachea of control chickens and an additional 6 ASVs were more abundant in the tracheal microbiota of contact chickens (Figure 4).

The inclusion of cecal samples in this analysis showed that a higher abundance of *P. mirabilis, F. plautii, B. argi*, [*Ruminococcus*] *torques* and *E. coli* in skin and tracheal microbiota of control chicks was affected by the ability of these species to colonize the caecum. Other bacteria, e.g., *N. subterraneum, S. hankookensis, C. acnes*, and *A. johnsonii*, due to their low abundance in the caecum, were likely of environmental origin (Figure 4).

Skin and tracheal microbiota in contact chickens was influenced by the transfer of gut microbiota from hens to chicks. This was likely true also for *Lactobacillus aviarius*, which colonizes mainly the small intestine and is represented in the caecum at low abundance. Contact hens acted as donors of *Lactobacilli* since *L. gallinarum* and *L. reuteri* were present in skin and tracheal microbiota of contact chicks at a significantly higher abundance than in control chicks. *S. pasteurianus* was the bacterium present in all skin samples and all but one tracheal samples but was sparsely present in the cecal samples of contact chicks indicating that this is a skin-specific bacterial species with transmission dependent on contact with adult hens (Figure 4). Contact with an adult hen also resulted in a significantly decreased abundance of *C. perfringens, E. coli* and *P. mirabilis* both on the skin and in the trachea of contact chicks.

## DISCUSSION

In this study, we evaluated the importance of contact between hens and chicks for the development of chicken skin and tracheal microbiota. Such contact is important for the development of gut microbiota (Kubasova et al., 2019; Marcolla et al., 2023) but consequences for the

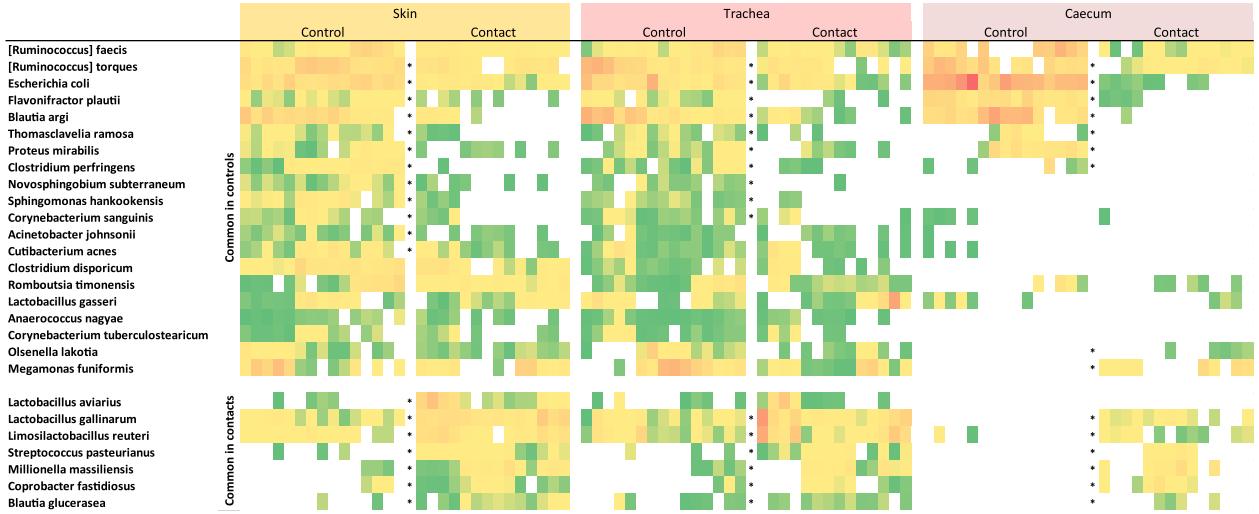


**Figure 4.** Major ASVs differentially abundant in skin and tracheal microbiota of control and contact chicks. Abundance of the most frequent ASVs was compared in skin and tracheal microbiota of control and contact chicks. Abundances in caeca were included to estimate the role of cecal colonization for each ASV. Columns represent individual chicks included in this study. Empty cell means that the ASVs was not recorded in the given sample. Green color indicates lowest abundance (0.0024% in this figure), yellow means intermediate abundance and red color represents the highest abundance. The highest abundance of 78.3% was recorded for *E. coli* in the caecum of one of the control chickens. Asterisks in free columns between control and contact chicks indicate a significant difference determined by Mann−Whitney test ($P < 0.05$). Abbreviation: ASVs: amplicon sequence variants.

development of skin and tracheal microbiota are not known.

Earlier studies showed that skin microbiota of broilers at slaughterhouses contained *E. coli, E. faecalis, Staphylococcus lentus*, different *Lactobacillus* species, *P. faecium*, and *Bacteroides dorei* (Hinton and Ingram, 2000; Oakley et al., 2013). Human skin is colonized by bacteria from genera *Staphylococcus, Cutibacterium* (earlier *Propionibacterium*) and *Corynebacterium* (Grice and Segre, 2011; Flowers and Grice, 2020). Staphylococci, Streptococci, Enterococci, and Corynebacteria together with [*Ruminococcus*] *torque, Blautia, E. coli* and *Phascolarctobacterium* belong to regular bacterial species also in the respiratory tract (Johnson et al., 2018; Wang et al., 2020a; Zhou et al., 2021). All these genera and species were recorded in skin and tracheal microbiota also in this study. Unlike previous reports, we did not detect *Brevibacterium, Brachybacterium* or *Xanthomonas* common in the litter (Kubasova et al., 2022) that were detected in chicken tracheal microbiota (Sohail et al., 2015; Johnson et al., 2018; Taylor et al., 2020), likely due to the short experiment duration and clean environment of the animal house used. On the other hand, we recorded alternative species of environmental origin like *Sphingomonas* or *Novosphingobium* (Bizjak et al., 2023; Shi et al., 2023). The origin of *Blautia*, [*Ruminococcus*] *torques, Succinatimonas* or representatives of *Selenomonadaceae, Bacteroidaceae or Rikenellaceae* was likely from the intestinal tract, in agreement with previous reports showing that Firmicutes dominated in the respiratory tract of young chickens (Wang et al., 2022) while adult hen lung microbiota contained an increased amount of Bacteroidetes (Van Goor et al., 2020). Unlike previous studies, we recorded representatives of the family Clostridiaceae (*C. perfringens* and *C. disporicum*) and Oxalobacteraceae (*Janthinobacterium violaceinigrum*). Of these, *C. disporicum, E. coli*, [*Ruminococcus*] *torques* and different *Lactobacilli* we recently proposed as bacteria widely associated with poultry production (Rychlik et al., 2023).

Despite overall similarities between skin and trachea microbiota, there were several differences. *C. perfringens, C. disporicum*, and *Staphylococcus equorum* were significantly less abundant in the trachea than in the skin. The respiratory tract was also poorly colonized by Enterococcaceae, Staphylococcaceae, *Sphingomonas* or *Novosphingobium, A. johnsonii, R. timonensis* and *Turicibacter bilis*. Of these, only Staphylococci may represent skin-adapted microbiota since *C. disporicum* is a broadly distributed taxon (Rychlik et al., 2023), *C. perfringens* can colonize the intestinal tract (Volf et al., 2021), *Sphingomonas* or *Novosphingobium* are plant rhizosphere microbiota (Bizjak et al., 2023; Shi et al., 2023) and the origin of *R. timonensis* and *Turicibacter bilis* is likely in the small intestine (Rychlik, 2020).

Contact of newly hatched chicks with adult hens considerably affected skin and trachea microbiota composition. *C. perfringens, E. coli, A. johnsonii* or *P. mirabilis*, all potential pathogens, were less abundant in skin microbiota of contact than control chicks suggesting a positive role of contact hen presence for chick skin and respiratory tract health status. Contact with an adult hen resulted in an increased abundance of different *Lactobacillus* species and these, together with *S. pasteurianus*, may be considered as potential probiotics for respiratory tract colonization. Skin and tracheal microbiota of contact chicks was enriched also for *Phascolarctobacterium, Succinatimonas* and *Turicibacter*. Since these species are strict anaerobes surviving air exposure for less than 24 h (Medvecky et al., 2018), these bacteria likely did not multiply in the skin or trachea and their DNA only temporarily persisted on the skin and in the

respiratory tract. However, even LPS and other microbe-associated molecular patterns from nonviable bacteria from the intestinal tract may stimulate the chicken innate immune system and contribute to an increased resistance to pathogens in the respiratory tract.

## CONCLUSIONS

Contact of newly hatched chicks with adult hens considerably affected skin and trachea microbiota composition. Contact with an adult hen resulted in an increased abundance of different *Lactobacillus* species as well as *S. pasteurianus* and a decreased abundance of *C. perfringens, E. coli, A. johnsonii* or *P. mirabilis*. It is possible that probiotic *Lactobacilli* intended for intestinal tract colonization instead increase chicken resistance to respiratory disorders, especially when we showed recently that *Lactobacilli* do not colonize chicken intestinal tract efficiently and do not protect chicks against *Salmonella* infection (Juricova et al., 2022). *Lactobacilli* together with *S. pasteurianus* may be considered as potential probiotics for respiratory tract colonization.

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## DISCLOSURES

The authors declare that they have no competing interests.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103302.

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