

# Identification of bacteria in the tracheal swabs of farmed ostriches and their effect on the viability of influenza A virus

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## Abstract

Avian influenza surveillance is a requirement for commercial trade in ostrich products, but influenza A viruses (IAVs) have proven difficult to isolate from ostrich tracheal swabs that test positive using molecular methods. We hypothesized that microbes unique to the ostrich trachea propagate in the transport medium after sampling and affect viral viability. We cultured tracheal swabs from 50 ostriches on 4 farms in South Africa, and recovered and identified 13 bacterial, 1 yeast, and 2 fungal species. *Dietzia* sp. had not been identified previously in the oropharyngeal tract of a bird, to our knowledge. The bacteria were tested for antimicrobial susceptibility, and most aerobic species, except for *Streptococcus* sp. and *Pseudomonas* sp., were sensitive to enrofloxacin; all were susceptible to sulfonamide. Virus inhibition experiments determined that ostrich-source *Streptococcus* sp., *Pantoea* sp., and *Citrobacter freundii* produced extracellular metabolites that caused a substantial reduction in the IAV titers of 99.9%. *Streptomyces*, *Corynebacterium*, *Staphylococcus*, *Arthrobacter gandavensis*, *Pseudomonas putida*, and *Acinetobacter* spp. similarly reduced the viability of IAV from 77.6% to 24.1%. *Dietzia* appeared to have no effect, but *Rothia dentocariosa*, *Rhodotorula* spp., and *Clostridium* spp. slightly increased the viability of IAV by 25.9, 34.9, and 58.5%, respectively.

**Keywords:** antimicrobial resistance; antiviral; influenza A virus; ostrich; tracheal swabs.

Commercially farmed ostriches (*Struthio camelus*) are highly versatile production animals valued for their fine leather, feathers, and low-cholesterol lean red meat. Ostriches are native to South Africa and thrive in the semi-desert climate of the areas of the southern Cape Provinces where the farms are concentrated, but the extensive nature of ostrich farming results in the transmission of influenza A viruses (IAVs) from the excretions of wild birds in the environment on a regular basis.<sup>1,2</sup> South Africa is the leading exporter of ostrich products globally, with 75% of the market share; the European Union is the main market.<sup>5</sup> To comply with the strict international trade requirement to ensure freedom from avian influenza, all

registered export farms are tested monthly, pre-movement, and pre-slaughter. Birds in epidemiologic units that test seropositive are re-sampled within 7 d, and tracheal swabs are collected for virus detection. An epidemiologic unit is defined as any group of ostriches of similar age and/or weight, with a defined epidemiologic relationship that share approximately the same likelihood of exposure to a pathogen, either because of sharing a common environment or because of common management practices. Tracheal swabs are also collected from each epidemiologic unit of all ostrich farms in the country twice a year for compulsory IAV surveillance.

Ostrich tracheal swab fluids are tested for the presence of IAV-specific RNA with reverse-transcription real-time PCR (RT-rtPCR) assays. The swab fluids of positive pools are then treated with antimicrobials and inoculated into embryonated chicken eggs (ECEs) for virus isolation, the international gold standard for virus identification.<sup>19</sup> There is a statistical correlation between the cycle threshold (Ct) value of an RT-rtPCR-positive sample and the ability to isolate the virus in ECEs,<sup>5,12</sup> but even with Cts <30, the success rate for isolation of IAVs from ostriches has been consistently low.<sup>1-3</sup> For example, in 977 ostrich tracheal swab pools tested by a national veterinary laboratory in 2019 using RT-rtPCR, 109 (11.2%) were IAV positive, but only one virus, an H1N1 strain, was isolated (Olivier A, pers. comm., unpublished diagnostic test reports, 2019). During the 2011 highly pathogenic avian influenza (HPAI) H5N2 subtype outbreaks in ostriches, only 3 viruses were isolated from >1,000 swab pools of 20 commercial ostrich farms. In the 2017 HPAI H5N8 subtype outbreaks, 38 ostrich tracheal swab pools were H5N8 positive on RT-rtPCR, but only 2 viruses were isolated compared with 39 viruses isolated from the oropharyngeal and cloacal swabs or tissue samples of other avian species.<sup>3</sup>

The consistently low rate of IAV isolation from ostriches is of concern to national and international regulators, and it is notable that the standard PBS-glycerol transport medium used routinely for ostrich tracheal swabs does not contain any antimicrobials. To investigate why IAVs in ostrich tracheal swab fluids are difficult to isolate in ECEs, we cultured and identified the bacteria present, tested their susceptibility to commonly used antimicrobials, and evaluated their effects on the viability of IAV.

## **Materials and methods**

### **Sample collection**

Ten randomly selected swab pools ( $n = 50$  ostriches; 5 per pool) collected in April 2018 from 4 geographically separated ostrich farms in the Western Cape Province that tested IAV-negative during routine surveillance at the Western Cape Provincial Veterinary Laboratory (WCPVL; Stellenbosch, Western Cape Province, South Africa), and had been stored at 4°C since receipt 48 h after collection, were shipped on ice packs to the University of Pretoria (UP; Onderstepoort, South Africa). Pools consisted of 5 rayon-tipped swabs each in 50% v/v glycerol PBS, pH 7.0–7.4, without antimicrobials.

### **Microbial culture and identification**

Ostrich tracheal swabs were removed aseptically from the transport medium and streaked onto various agar plates for bacterial isolation. For aerobic bacteria, Columbia blood agar with 5% horse blood (Thermo Fisher) and MacConkey agar without crystal violet (Thermo Fisher) were incubated at  $37 \pm 1^\circ\text{C}$  with or without 5%  $\text{CO}_2$ , respectively, for 24 h. For

anaerobic bacteria, Columbia blood agar plates with 5% horse blood were incubated at  $37 \pm 1^\circ\text{C}$  for 24 h in an anaerobic incubator. Plates were examined every 24 h, for up to 72 h. Where necessary, bacterial colonies were subcultured using a quadrant streaking method to obtain axenic cultures.<sup>7</sup> Potato dextrose agar (Oxoid) and Dermasel agar plates (Oxoid) were used to isolate fungal species, with incubation at  $25 \pm 1^\circ\text{C}$  for up to 1 mo. Subculturing to obtain axenic fungal cultures was performed where required. Gram staining was applied for the preliminary identification of axenic bacterial cultures, and lactophenol cotton blue stain was used for fungal species identification, using standard procedures for both.<sup>10</sup> Catalase and oxidase tests were performed according to standard methods in the preliminary identification of bacteria,<sup>10</sup> and were supplemented with API 10 S strips (bioMérieux) for the identification of the Enterobacteriaceae and other non-fastidious gram-negative bacteria that were oxidase negative. API 20 NE strips (bioMérieux) were used for the identification of non-fastidious, non-enteric gram-negative rods and gram-negative bacteria that were oxidase positive and grew poorly on the MacConkey agar. Rapid ID 32 A strips (bioMérieux) were used for the identification of anaerobes. Streptococcal species were tested using the Lancefield streptococcal grouping kit (Oxoid). API 20 C Aux (bioMérieux) was used in the identification of isolated yeasts. All tests were performed according to the kit-recommended procedures. Carbohydrate fermentation tests were used to identify *Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp., and *Micrococcus* spp., as prescribed.<sup>10</sup> Furthermore, suspected *Staphylococcus* spp. that were gram-positive cocci, oxidase negative, and catalase positive were tested with the DNase + mannitol test and the purple agar with maltose test. An antimicrobial susceptibility test was performed with the novobiocin disk (Oxoid) and the polymyxin B disk (Oxoid) on a Mueller–Hinton agar plate (Thermo Fisher) to confirm *Staphylococcus* spp. The plates were incubated for 24 h at  $37 \pm 1^\circ\text{C}$ , after which all reactions were recorded. Differentiation between *Staphylococcus* and *Micrococcus* species was also possible by measuring the zone of a bacitracin disk (Oxoid). Samples were not cultured for mycoplasma. Bacterial isolates were inoculated aseptically into cryovials containing 1.5 mL of BHI broth (Oxoid), mixed by vortexing, and frozen; isolates were then stored at  $-80^\circ\text{C}$  until further tested.

Axenic aerobic cultures streaked onto agar plates were submitted to NOSA Testing (Pretoria, South Africa) for bacterial identification by MALDI-TOF mass spectrometry (Microflex; Bruker). Scores of 2.00–3.00 represented high confidence identification, scores of 1.70–1.99 represented low confidence identification, and no identification was possible when scores were 0.00–1.69.

### **Antimicrobial susceptibility tests**

The standardized Kirby–Bauer disk diffusion method was used to determine the antimicrobial susceptibility of the axenic bacterial isolates. Cultures stored at  $-80^\circ\text{C}$  were reinoculated into microbiologic media. For the aerobic isolates, antibiograms were conducted against the standard antimicrobials used in the poultry industry, namely ampicillin, colistin sulfate, doxycycline, enrofloxacin, erythromycin, gentamicin–neomycin, sulfonamide–trimethoprim, tetracycline, ceftiofur (all sourced from Oxoid) and fosfomicin (Fosbac). Clinical resistance breakpoints in Suppl. Table 1 were as per Clinical Laboratory Standards Institute guidelines.<sup>4</sup>

### **16S rRNA sequencing**

Bacterial cells were pelleted from 5 mL of culture medium by centrifugation at  $10,000 \times g$  for 10 min (5804R; Eppendorf). Genomic DNA was extracted from the pellets (Invitrogen

**Table 1.** Identification of microbial species cultured from ostrich tracheal swab pools.

Isolate	Classical phenotypic methods	MALDI-TOF mass spectrometry		16S rRNA sequence		
		Identity	Score	Closest match in GenBank	Accession	% identity
B700/18-0	<i>Clostridium</i> sp.*	Not tested		<i>Clostridium</i> sp.	KT592379	100
B700/18-1	<i>Micrococcus</i> sp.*	<i>Dietzia maris</i>	1.90	<i>Dietzia maris</i>	MG547917	98.6
B700/18-2	<i>Acinetobacter</i> sp.†	<i>Acinetobacter lwoffii</i>	2.08	<i>Acinetobacter</i> sp.	MK548512	99.9
B700/18-3	<i>Brevibacterium</i> sp.*	<i>Arthrobacter gandavensis</i>	2.43	<i>Arthrobacter gandavensis</i>	MK646035	99.7
B700/18-5	<i>Streptococcus equinus</i> *	Unidentified	1.55	<i>Streptococcus pluranimalium</i>	CP025536	98.1
B700/18-8	<i>Pantoea</i> sp.*	Not tested		<i>Pantoea septica</i>	HM854257	98.6
B700/18-9	<i>Vibrio parahaemolyticus</i> †	<i>Pseudomonas putida</i>	1.86	<i>Pseudomonas putida</i>	CP024086	99.8
B700/18-10	<i>Citrobacter freundii</i> †	Unidentified	1.91	Not sequenced		
B700/18-15	<i>Rothia dentocariosa</i> *	<i>Rothia dentocariosa</i>	2.30	Not sequenced		
B700/18-16	<i>Corynebacterium bovis</i> *	Unidentified	1.60	<i>Corynebacterium falsenii</i>	KT824798	98.3
B700/18-17	<i>Streptococcus</i> sp.*	Unidentified	1.40	Uncultured bacterium	AB989765	99.3
B700/18-18	<i>Streptomyces</i> sp.*	<i>Streptomyces badius</i>	1.74	<i>Curtobacterium</i> sp.	HF566227	96.7
B700/18-19	<i>Staphylococcus</i> sp.*	<i>Staphylococcus haemolyticus</i>	2.08	<i>Staphylococcus haemolyticus</i>	CP033814	99.8
B700/18-21	<i>Rhodotorula mucilaginosa</i> ‡	Not tested		Not sequenced		
B700/18-22	<i>Purpureocillium lilacinum</i> §	Not tested		Not sequenced		
B700/18-23	<i>Alternaria</i> spp.§	Not tested		Not sequenced		

\*Gram-positive bacterium.

†Gram-negative bacterium.

‡Yeast.

§Fungi.

|DNA was below the quality cutoff for barcoded library preparation.

**Table 2.** Antimicrobial susceptibility of aerobic bacteria cultured from ostrich tracheal swabs.

Isolate	Species	Ampicilin (10 µg)	Colistin sulfate (10 µg)	Doxycycline (30 µg)	Enrofloxacin (15 µg)	Erythromycin (15 µg)	Fosfomicin (200 µg)	Ceftiofur (30 µg)	Neomycin (10 µg)	Kanamycin (30 µg)	Sulfonamides (300 µg)	Trimethoprim (25 µg)	Tetracycline (30 µg)
B700/18-1	<i>Dietzia</i> sp.	S	<b>R</b>	—	S	S	<b>R</b>	S	—	I	S	<b>R</b>	S
B700/18-2	<i>Acinetobacter</i> sp.	S	S	S	S	—	—	I	S	S	S	S	S
B700/18-3	<i>Arthrobacter gandavensis</i>	—	<b>R</b>	—	S	—	—	S	S	S	S	S	S
B700/18-5	<i>Streptococcus</i> sp.	S	<b>R</b>	—	I	S	—	S	—	<b>R</b>	S	S	I
B700/18-8	<i>Pantoea</i> sp.	<b>R</b>	S	S	S	—	—	S	S	S	S	S	S
B700/18-9	<i>Pseudomonas putida</i>	—	S	—	I	—	—	<b>R</b>	S	S	S	I	S
B700/18-10	<i>Citrobacter</i> sp.	<b>R</b>	S	S	S	—	—	S	S	S	S	S	S
B700/18-15	<i>Rothia dentocariosa</i>	—	<b>R</b>	—	S	S	—	S	—	I	S	S	S
B700/18-16	<i>Corynebacterium</i> sp.	—	<b>R</b>	—	S	S	—	S	—	S	S	S	S
B700/18-18	<i>Streptomyces</i> sp.	—	S	—	S	—	—	<b>R</b>	—	<b>R</b>	S	S	I
B700/18-19	<i>Staphylococcus haemolyticus</i>	S	<b>R</b>	—	S	S	—	S	—	S	S	S	S

I = intermediate; R = resistant (in bold); S = sensitive; — = susceptibility testing is not recommended; the species is a poor target for therapy with the agent.

PureLink genomic DNA mini kit; Life Technologies, Thermo Fisher) using the protocols for gram-positive or gram-negative bacteria. DNA was eluted in 50  $\mu$ L of ultrapure water and quantified (NanoDrop spectrophotometer; Inqaba Biotech). DNA samples were supplied to the Ion Torrent PGM sequencing service facility at the University of Pretoria, where 100 ng of each sample was used to build 400-bp barcoded libraries (Ion 16S metagenomics kit; Thermo Fisher), according to the recommended procedure. 16S barcoded libraries were shipped to Stellenbosch University's Central Analytical Facility (Stellenbosch, South Africa), where massively parallel sequencing was performed (S5-530 chip, Ion Proton system; Thermo Fisher) according to the recommended procedures. Ion Torrent sequencing results were analyzed (CLC Genomics Workbench v.5.1.2; Qiagen). Contigs assembled de novo were identified by BLAST sequence homology (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Sequences generated were deposited in GenBank as MN814254–MN814263.

## Virus inhibition

Low pathogenic influenza virus A/ostrich/South Africa/OU2/2012 (H7N1) was propagated in 9- to 11-d-old specific pathogen-free ECEs and titrated using the method of Reed and Muench.<sup>14</sup>

A single colony of each bacterium was inoculated into 10 mL of sterile BHI broth and grown overnight with shaking in a 37°C incubator. Cultures were centrifuged at 5,000  $\times$  g for 10 min to pellet the cells, and the medium was aspirated and passed through 0.45- $\mu$ m syringe filters (GVS Life Sciences) into sterile containers. To test whether the filtered overnight cultures were toxic to the chicken embryos, 0.2-mL volumes of each sample were inoculated into 6 ECEs each and incubated at 37°C for 7 d. No embryo deaths were recorded.

Filtered BHI broth (2.25 mL) containing the bacterial metabolites plus a control of sterile BHI broth were incubated with 0.25 mL of egg allantoic fluid containing 10<sup>9.09</sup> EID<sub>50</sub> live A(H7N1) virus for 1 h at 37°C. Each sample was titrated from 10<sup>-1</sup> to 10<sup>-10</sup> in PBS, and at least 4 titrations of each sample was inoculated into 6 ECEs (Suppl. Table 2). Eggs were incubated for 6 d and inspected daily for deaths. Any deaths occurring prior to day 3 were considered nonspecific. The final virus titer in any eggs that died from day 3 onwards was calculated according to Reed and Muench.<sup>14</sup>

## Results

### Identification of bacteria isolated from ostrich tracheal swabs

Thirteen different bacteria, 1 yeast, and 2 fungal species were isolated and identified by classical techniques from tracheal swabs (Table 1). MALDI-TOF MS confirmed the identification of most bacteria, but there were some discrepancies between the results, for example, B700/18-3 *Brevibacterium* sp. was identified by MALDI-TOF MS as *Arthrobacter gandavensis*, therefore 16S rRNA sequencing was used for definitive identification. Two samples, B700/18-10 and B700/18-15, were below the DNA quality cutoff for barcoded DNA library construction and were therefore not sequenced. The species identification was determined by a consensus of at least 2 methods and/or sequence homology >99% on 16S rRNA. Thus, the following microbes were identified—gram-positive bacteria: *Clostridium* sp., *Dietzia* sp. (although *D. maris* was identified by 2 methods; the MALDI-TOF score was only 1.90, and the 16S rRNA sequence homology was 98.6%), *A. gandavensis*, 2 *Streptococcus* spp., *Pantoea* sp., *Rothia dentocariosa*, 2 *Streptomyces* spp., and *Staphylococcus haemolyticus*; gram-negative bacteria: *Acinetobacter* sp., *Pseudomonas*

*putida*, and *Citrobacter* sp.; yeast: *Rhodotorula mucilaginosa*; fungi: *Purpureocillium lilacinum* and *Alternaria* sp. (Table 1).

The aerobic bacteria were tested for susceptibility against different antimicrobial classes commonly used in poultry, namely doxycycline (100 mg/L; class tetracycline), enrofloxacin (100 mg/L; class quinolones), and penicillin–streptomycin (100 mg/L; class penicillin). B700/18-17 (*Streptococcus* sp.) could not be revived after storage at  $-80^{\circ}\text{C}$  and was thus not tested. Of the 3 bacteria for which doxycycline breakpoints are available (*Acinetobacter* sp., *Pantoea* sp., *Citrobacter* sp.), all were susceptible to 30  $\mu\text{g}$  (Table 2). Most aerobic bacterial species were sensitive to 15  $\mu\text{g}$  of enrofloxacin, except for *Streptococcus* sp. and *Pseudomonas* sp., which were of intermediate susceptibility. Penicillin–streptomycin was not in the antibiogram panel; however, *Dietzia*, *Acinetobacter*, and *Streptococcus* spp. were susceptible to ampicillin. All aerobic species were susceptible to 300  $\mu\text{g}$  of sulfonamide.

Colistin is effective against most gram-negative bacilli, but most gram-positive bacilli are resistant, as are gram-positive aerobic cocci, gram-positive aerobic bacilli, and all anaerobes.<sup>7</sup> All 4 gram-negative aerobic bacteria (*Acinetobacter* sp., *Pantoea* sp., *P. putida*, *Citrobacter* sp.) isolated from ostriches were susceptible to 10  $\mu\text{g}$  of colistin sulfate, as expected. Most of the gram-positive aerobic bacteria identified (*Dietzia* sp., *Arthrobacter* sp., *Streptococcus* sp., *R. dentocariosa*, *Corynebacterium* sp., *S. haemolyticus*) were indeed resistant to colistin, but the gram-positive *Streptomyces* sp. isolated from ostriches was susceptible.

The single anaerobic isolate (B700/18-0), a *Clostridium* sp., was tested with a MASTRING-S test (Mast Group). The isolate was sensitive to erythromycin (60  $\mu\text{g}$ ), rifampicin (15  $\mu\text{g}$ ), penicillin G (2 units), kanamycin (100  $\mu\text{g}$ ), and vancomycin (5  $\mu\text{g}$ ), but was resistant to colistin sulfate (10  $\mu\text{g}$ ).

### **Effect of bacteria-free growth media on the viability of influenza A virus**

Titration of A(H7N1) virus were incubated with the filtered overnight bacterial cultures, and the treated virus was inoculated into eggs for virus isolation alongside controls incubated in sterile BHI medium (Suppl. Table 2). Three of the bacterial species, namely *Streptococcus*, *Pantoea*, and *C. freundii*, caused a substantial reduction in the viability of the A(H7N1) virus, with 99.9% reductions in the viral titers compared to the controls (Fig. 1). *Streptomyces*, *Corynebacterium*, *Staphylococcus*, *A. gandavensis*, *P. putida*, and *Acinetobacter* spp. similarly reduced the viability of IAV by 77.6% to 24.1% compared to the controls. *Dietzia* appeared to have no effect, but *Rothia*, *Rhodotorula*, and *Clostridium* spp. slightly increased the viability of the virus by 25.9, 34.9, and 58.5%, respectively.

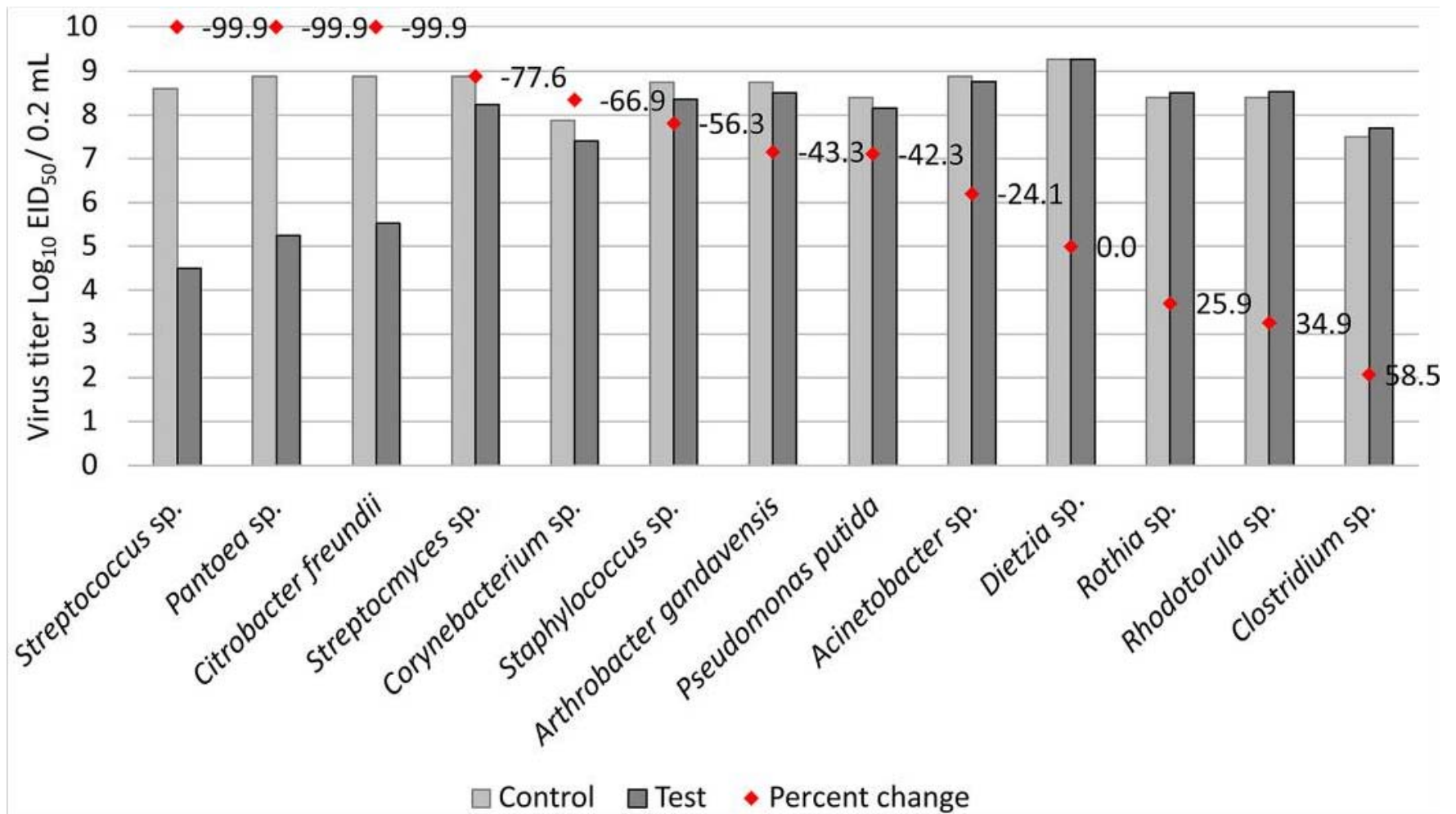


Figure 1. Effect of bacteria isolated from ostrich tracheal swabs on the viability of influenza A virus.



## Discussion

Five of the 16 microbes cultured from ostrich tracheal swabs were described as colonizing agents of the ostrich oropharynx in a study conducted on 50 healthy breeder ostriches in Brazil.<sup>11</sup> That study identified only 2 gram-positive species, namely *Staphylococcus* sp. and *Streptococcus* sp., whereas 12 gram-negative strains were identified.<sup>11</sup> Of the latter, only *Citrobacter* sp. and *Pseudomonas* sp. were identified in our study. *Rhodotorula* was also identified previously, but we isolated neither *Candida albicans* nor *Cryptococcus* spp.

Interestingly, we identified a *Dietzia* sp. by MALDI-TOF MS and confirmed its identity by 16S rRNA sequencing. *Dietzia* are gram-positive, aerobic, non-spore-forming bacteria taxonomically closely related to actinomycetes, with *D. maris* originally described as *Rhodococcus maris*.<sup>13</sup> *Dietzia* strains inhabit diverse environments, including the oropharyngeal tracts of dogs,<sup>6</sup> but *Dietzia* has not been cultured from or identified in the oropharyngeal tract of a bird previously, to our knowledge.

Microbes produce and secrete extracellular metabolites into their environment during their growth. These may include primary metabolites such as hydrophilic carbohydrates, volatile alcohols, ketones, amino and non-amino organic acids, hydrophobic lipids, and complex secondary metabolites such as antibiotics, pigments, non-ribosomal peptides, and cofactors. The production and secretion of these primary and secondary metabolites is complex and variable, dependent on factors such as temperature, pH, and the availability and concentration of nutrients.<sup>17</sup> Rhamnolipids, for example, are secondary metabolites produced by *Pseudomonas* spp. as well as by *D. maris*. Rhamnolipids are very efficient biosurfactant molecules that are harnessed for industrial applications, for example in the bioremediation of oil spills,<sup>9,18</sup> but rhamnolipids have also been demonstrated to have antiviral activity against enveloped viruses.<sup>8</sup> In another example, an 11-kDa protein isolated from the cyanobacterium *Nostoc ellipsosporum* was demonstrated to have antiviral activity against human immunodeficiency virus and IAV, through binding to viral surface glycoproteins.<sup>15</sup>

Ostrich tracheal mucus and saliva on swabs are rich sources of proteins, enzymes, carbohydrates, and electrolytes in the PBS-glycerol transport medium. We found that ostrich tracheal swab fluid supports the growth of at least 16 different microbes, and that most of the bacteria present produce extracellular metabolites that have substantive antiviral effects against IAV. The disruption of the IAV envelope by bacterial proteins would render the virus nonviable, although the viral RNA might still be intact. This could explain why ostrich IAVs are easily detectable by RT-rtPCR but are not isolatable in chicken eggs. A limitation of our study is that we did not determine the prevalence of each of the microbes in the ostrich tracheal swab samples, and the identification of the antiviral compounds was beyond the scope of our investigation.

Some of the bacteria we isolated and identified were resistant to some of the antimicrobial compounds tested, but sulfonamide used at 300 µg appeared to be a suitable broad-spectrum antimicrobial to include in a viral transport medium. Colistin resistance is a worldwide medical concern given that it is a last-line treatment for carbapenemase-producing bacteria. Six of the 7 gram-positive aerobic bacteria that we identified in ostriches, namely *Dietzia* sp., *Arthrobacter* sp., *Streptococcus* sp., *R. dentocariosa*, *Corynebacterium* sp., and *S. haemolyticus*, were resistant to colistin sulfate.

Antimicrobials are not used as growth promoters in ostriches, as is common in traditional poultry production, but are administered to treat specific infections, for example quinolones in chicks for yolk sac infections, and sulfonamide, oxytetracycline, tylosin, and fosfomycin for intestinal infections. Lincomycin–spectinomycin is used for intestinal and respiratory infections, and penicillins are used for intestinal infections. In grower birds (4–12 mo old), oxytetracycline, tylosin, and penicillins are used to treat respiratory and intestinal infections, but colistin has not been used in ostriches in over a decade (Olivier A, pers. comm., 2020).

The World Organisation for Animal Health (OIE) specifically recommends that antibiotics should be added to viral transport media for IAV screening,<sup>19</sup> but this has not been a standard practice in South Africa. Other studies report that PBS should be avoided as a transport medium when virus isolation will be performed because the virus is less stable in PBS, which significantly reduces the sensitivity of isolation compared to a protein-buffered medium.<sup>16</sup> Further studies should investigate the inclusion of broad-spectrum antimicrobials such as sulfonamide and enrofloxacin in a protein-based transport medium for swabs to assess whether virus isolation rates from ostriches can be improved.

## **Acknowledgments**

We thank Nicky Olivier, Renate Zipfel, and Dionne Rauff for technical assistance.

## **Declaration of conflicting interests**

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

## **Funding**

Our study was supported by the South African Department of Science and Technology/National Research Foundation’s South African Research Chair Initiative under grant 114612, the Department of Trade and Technology–funded “Healthy Flocks-Quality Leather” grant, and the Western Cape Department of Agriculture. Reneé Pieterse and some of the testing costs were funded from the Belgian Directorate-General for Development Co-operation Framework Agreement (FA4 DGD-ITM 2017-2021) grant FA4-N02000.

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