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Technical Note

Raoultella ornithinolytica Isolation in Cloacal Microbiota of Tinamus solitarius: Preliminary Data

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ABSTRACT

Raoultella ornithinolytica is a gram-negative aerobic bacterium belonging to Enterobacteriaceae family, an emerging pathogen that causes several pathogenic conditions in man, with little veterinary importance; however, its identification is underestimated by conventional laboratory techniques. The present study reports the identification of *R. ornithinolytica* in *Tinamus solitaries*, during a routine sanitary evaluation of aerobic enterobacteria in cloacal microbiota of birds belonging to the Güira Oga Center, Puerto Iguazu, Argentina. The sample was preliminary classified as *Klebsiella* spp.; however, after the use of the MALDI-TOF MS technique it was identified as *R*. o*rnithinolytica*. The sample was submitted to an antimicrobial susceptibility test, where it showed a similar pattern profile as reported in the literature, with resistance to ampicillin and other β-lactam antibiotics. It is possible that *Raoultella* spp are more common in birds as it is reported. Therefore, review studies on bacteria collections of avian origin, as well as cases with confirmation of *Klebsiella*, should be deeply evaluated in laboratorial routine, mainly due to the pathogenic potential of *R*. o*rnithinolytica* for Poultry, as well as for public health.

INTRODUCTION

Raoultella ornithinolytica is a gram-negative aerobic bacterium belonging to the Enterobacteriaceae family is commonly present in the aquatic environment (Park *et al*., 2011; Jong *et al*., 2013). The genus *Raoultella* are composed by species belonging to the genus *Klebsiella,* differentiated by molecular techniques and by having a distinct behavior such as growing at low temperatures, and by using sorbose as carbon source (Drancourt *et al*., 2001).

It is an emerging pathogenic bacterium that presents some virulence factors such as capsule, CFA/I and CFA/II colonization factors, as well as production of siderophores, histamine and bacteriocins (Podschun *et al*., 1993; Podschun *et al*., 1998; Kanki *et al*., 2007; Al-Hulu *et al*., 2009; Seng *et al*., 2016).

Due to the similarities between *Raoultella* and *Klebsiella* genus it is impossible to distinguish both by conventional biochemical techniques (de Jong *et al*., 2013), being identified only by advanced identification techniques.

The present study reports the identification of *Raoultella ornithinolytica* sample in a healthy *Tinamus solitarius* during a sanitary evaluation of aerobic enterobacteria in routine cloacal microbiota from a group of twenty one birds, belonging to the Güira Oga Center, Puerto Iguazu, Argentina.

Samples were collected through the swab technique. Prior to the collection cloacal asepsis was performed (with alcohol 70°) in order

to avoid contamination. Material collection was performed individually by using sterile swabs. Swabs were inserted in the cloaca through soft circular movements in order to maximize the contact with the cloacal mucosa. After collection, each swab was conditioned in flasks containing Stuart media and kept refrigerated at 8 °C until processing.

The samples were processed in the Laboratory of Microbiology at Universidade do Oeste de Santa Catarina (UNOESC, Xanxerê, SC, Brazil) where the swabs were incubated in peptone water at 40 °C for 24 hours. One mL of each peptone broth samples was added to tubes containing Brain and Heart Broth (BHI) and Tetrathionate Broth (TB), incubated again at 40°C during 24 hours. Then, the samples were seeded on MacConkey and Xylose-Lysine-Deoxycholate agar, incubated at 40 °C for 24 hours. All the different colonies that grew in the different media were selected and, after isolation of each strain, frozen at -80 °C in nutrient broth plus 10% glycerol and sent to the Ornithology Laboratory of Faculdade de Medicina Veterinária e Zootecnia of Universidade Estadual Paulista (FMVZ-UNESP, Botucatu, SP, Brazil) for the biochemical pre-screening assay of the samples, composed of urea, malonate broth, Sulfito-Indol-Motility medium, triple sugar and iron agar, and methyl red and Voges-Proskauer tests.

The samples were then sent to the Laboratório de Medicina Aviária of Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (FMVZ-USP, São Paulo, SP, Brazil), being submitted to the matrix assisted laser desorption technique - MALDI-TOF MS for confirmation of the isolated species.

The technique confirmed pre-identified samples in genus *Escherichia*, *Enterobacter*, *Serratia*, *Proteus* and *Klebsiella*; however, one of the pre-identified samples has proved to be *Raoultella ornithinolytica*.

R. ornithinolytica sample was subjected to anti-biogram sensitivity tests in order to assess the resistance profile, according to Bauer *et al*. (1966), using commercial antibiotic impregnated disks (Sensifar-Vet Cefar®, Cefar Diagnóstica Ltda, São Paulo, SP, Brazil). The sample was challenged by 26 distinct antibiotics (Table 1), demonstrating resistance to only seven antibiotics (ampicillin, bacitracin, enrofloxacin, erythromycin, spiramycin, penicillin and rifampicin) intermediate sensitivity only to amoxicillin.

R. ornithinolytica is known as one of the bacteria in charge for scombroid poisoning, a toxic condition due to the ingestion of fish with high levels of histamine (Masashi *et al.*, 2002); however, in the last decade it was considered as an emerging pathogen in humans (Seng *et al*., 2016) involved in urinary tract infection (García-Lozano *et al*., 2013; Nakasone *et al*., 2015), septicemia (Kaya *et al*., 2014; Sueifan *et al*., 2016), bacteremia (Mau & Ross, 2010; Hadano *et al*., 2012; Haruki *et al*., 2014; Sekowska *et al*., 2015), peritonitis (Sibanda, 2014), enteric fever (Morais *et al*., 2009), biliary infection (Cleveland *et al*., 2014; de Jong *et al*., 2014), infected "diabetic foot" (Solak *et al*., 2011; Kabbara & Zgheib, 2015) and artritis (Venus *et al*.,2016). However, cases of infection were always related to some factor of primary malignancy or immune deficiency (Chun *et al*., 2015; Boattini *et al*., 2016).

Table 1 – Antimicrobial susceptibility profile of *Raoultella ornithinolytica*.

Antibiotic	Concentration(µg)	Sensitivity*
Quinolones		
Nalidixicacid	30	S[1]
Ciprofloxacin	5	S
Enrofloxacin	5	R[2]
Norfloxacin	10	S
Tetracyclines		
Oxytetracycline	30	S
Tetracycline	30	S
Doxycycline	30	S
Aminoglycosides		
Amikacin	30	S
Streptomycin	10	S
Gentamicin	10	S
Neomycin	30	S
Macrolides		
Erythromycin	15	R
Spiramycin	100	R
Peptides		
Bacitracin	10	R
Colistin	10	S
Amphionicols		
Chloramphenicol	30	S
Penicillins		
Amoxicillin	10	I[3]
Amoxicillin + clavulanic acid	30	S
Ampicillin	10	R
Penicillin	10	R
Cephalosporins		
Cephalexin	30	S
Cephalothin	30	S
Ansamycins		
Rifampicin	5	R
Sulfonamides		
Sulfazotrim	25	S
Sulfonamide	300	S
Diaminopirimidinas		
Trimetropim	5	S

*Sensitivity criteria followed the Enterobacteria standard, as recommended by the manufacturer. [1]Sensitive; [2]Resistant; [3]Intermediate.

The isolation of this pathogen in animals is uncommon and there are few records in birds. As in the present case, it has already been described in a cloacal microbiota of a healthy vulture (Sala *et al*., 2016) and in a case of hepatitis in *Ring-Neck* (Gonzales-Lama & Lupiola-Gomez, 2007). Nevertheless, both cases were reported in Europe. Thus, this is the first report of *R. ornithinolytica* isolation in a wild bird in South America.

Regarding the antimicrobial susceptibility profile, as observed by Morais *et al.* (2009), Mau & Ross (2010), García-Lozano *et al*. (2013), Kaya *et al*. (2015), Sekowska *et al*. (2015), Ponce-Alonso *et al*. (2016) and Seng *et al*. (2016), there was low sensitivity to β-lactam antibiotics. This occurs due to the presence of chromosomal resistance genes for the production of β-lactamases (Walckenaer *et al*., 2004). The macrolide resistance observed in this study was also expected since, according to Ito et al. (2005), this pharmacological group is mainly indicated for Gram-positive bacteria and the efficiency is widely variable in Gram-negative bacteria. As mentioned in these studies, the resistance to other antibiotics are varied; thus, not showing a specific pattern.

In general, the incidence of *Raultella ornithinolytica* is underestimated due to the imprecision of conventional phenotypic identification methods, generating uncertainties as to its pathogenicity (Ponce-Alonso *et al*., 2016), mainly due to the similarities with species of the *Klebsiella* genus, such as *K. pneumonie* and *K. oxytoca*. The only way to achieve reliable identification of *R. ornithinolytica* and other species of *Raoultella* is applying supplemental biochemical tests and/or by using the MALDI-TOF MS technique.

It is possible that species of the genus *Raoultella* are common in birds. Cases of bacterial collection of avian origin, as well as the confirmation in cases involving samples *Klebsiella* in birds should be more investigated as potentially being *Raoultella*, in laboratorial routine, mainly due to its pathogenic potential for birds, as well as for public health. Therefore, it will be possible to determine the importance of birds in the epidemiological chain of this poorly studied bacterium.

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