



## MICROBIOLOGY

# Gulls as carriers of antimicrobial resistance genes in different biogeographical areas of South America

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**Abstract:** The aim of this communication was to establish if *Enterobacteriales* associated with gulls in Argentina harbored antimicrobial resistance (AMR) genes. We analyzed cloacal swabs in two contrasting areas: Ensenada, Buenos Aires province (26 *Larus dominicanus* and 22 *Chroicocephalus maculipennis*) and Puerto Madryn, Chubut province (20 *L. dominicanus*). In Ensenada,  $bla_{CTX-M}$  and *mcr-1* genes, were isolated from both gull species, whereas in the Puerto Madryn, only  $bla_{CTX-M}$  gene was found. We report for the first time *C. maculipennis* as carrier of AMR. The finding of AMR in wildlife constitutes a useful tool in evaluating the anthropogenic impact on environmental health.

**Key words:** Antimicrobial resistance, Argentina,  $bla_{CTX-M}$ , gulls, landfills, *mcr-1*.

## INTRODUCTION

Antimicrobial resistance (AMR) is the ability of microorganisms to grow at therapeutic concentrations of antibiotics (García-Hernández et al. 2011). Gram-negative bacilli (*Enterobacteriales*) extended-spectrum  $\beta$ -lactamases (ESBLs) enzymes production is a mechanism that confers resistance to broad-spectrum antimicrobials like cephalosporins. The CTX-M  $\beta$ -lactamase enzymes encoded by the  $bla_{CTX-M}$  gene, are the most worldwide distributed (Hernández et al. 2013, Darwich et al. 2019).

Colistin, antimicrobial polymyxin's family, was used until cephalosporins became available (Stein & Didier 2002). Since bacteria developed resistant to them, colistin was again used despite its toxicity (Sun et al. 2017). Recently, Liu et al. (2016) isolated the *mcr-1* plasmid-mediated

colistin resistance mechanism from *Escherichia coli*.

Wild birds can harbor and spread AMR locally or globally (Liakopoulos et al. 2016a). There are many studies about AMR in gulls from Europe (e.g. Bonnedahl et al. 2009, Simões et al. 2010, Vergara et al. 2016) however, in South America are very scant (Hernández et al. 2013, Liakopoulos et al. 2016a, b).

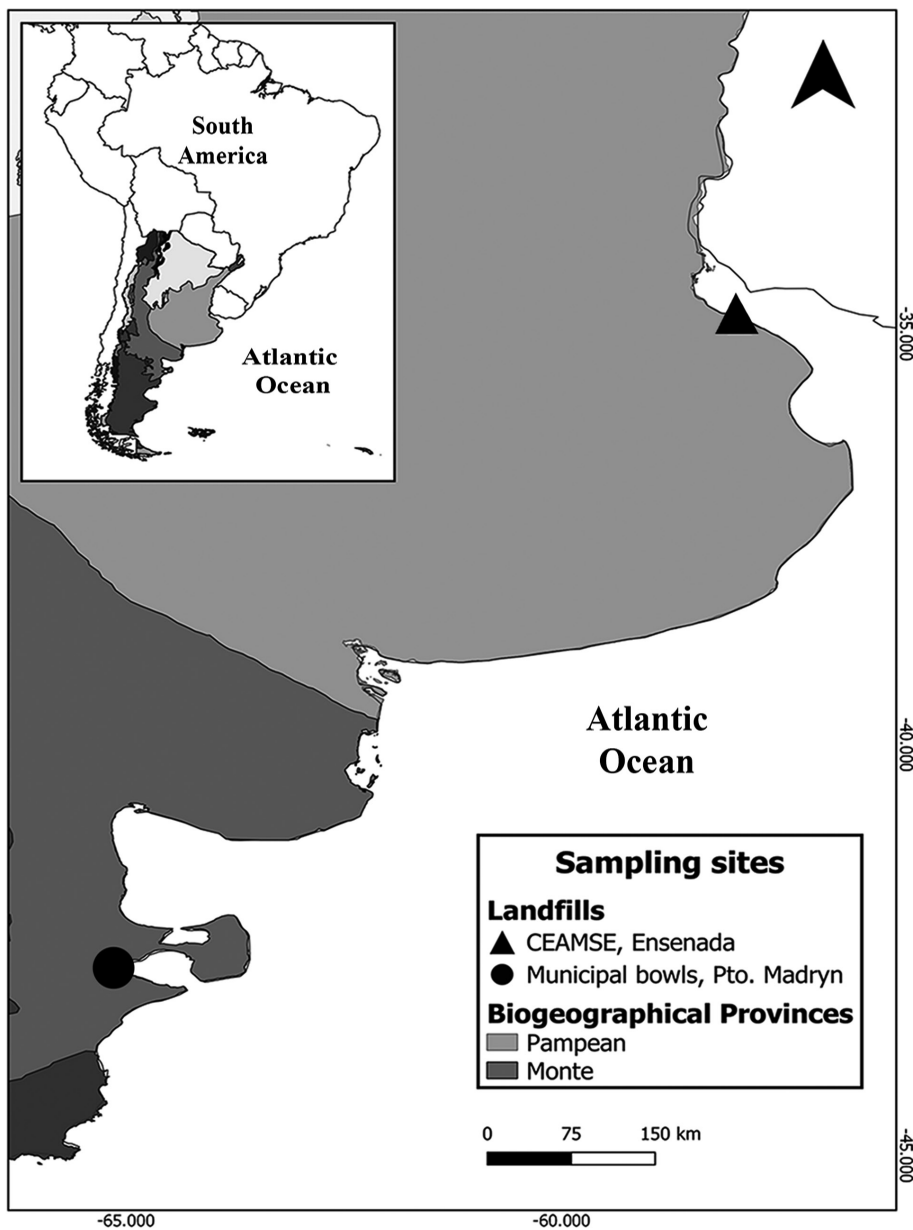
This study aimed to determine if *Enterobacteriales* associated to different gull species that visited landfills in two contrasting areas from Argentina, harbored  $bla_{CTX-M}$  and *mcr-1* genes, allowing us to understand if anthropogenic activities generate selective pressure on the environment, intervening in AMR presence on wildlife promoting its dissemination.

**MATERIALS AND METHODS**

**Study areas**

Two landfills were chosen because of their contrasting biogeographical location in South America, Argentina. One is a household waste sanitary landfill, administrated by CEAMSE (Coordinación Ecológica Área Metropolitana Sociedad del Estado) located in Ensenada city, Northeast Buenos Aires province, (34°51'13”S,

57°57'33”W) (Fig. 1). This site is in the Neotropical region, Pampean biogeographical province (Arana et al. 2017). The humid climate, the geochemical properties of the ground and the cycles of vegetation that characterize this region, promote agricultural activities (Burkart et al. 1999). Furthermore, Buenos Aires province concentrates a great human population density (50.8 hab/km<sup>2</sup>) ([https://www.indec.gob.ar/ftp/cuadros/poblacion/censo2010\\_tomo1.pdf](https://www.indec.gob.ar/ftp/cuadros/poblacion/censo2010_tomo1.pdf)).



**Figure 1. Sampling sites: landfills in each biogeographical province CEAMSE Ensenada, Pampean Province and Municipal Bowls Puerto Madryn, Monte Province.**

The other chosen site is an open landfill (Municipal bowls) located near Puerto Madryn city (Chubut province), on the northern Patagonian coast (42°43'52"S, 65°8'16"W) (Fig. 1). This area is part of the South American transition zone, in the biogeographical Monte province where the climate is temperate to arid and dries, and shrub vegetation is dominant (Arana et al. 2017). Due to that, agricultural activity is not developed as the previous region (Burkart et al. 1999). In addition, Chubut province has a lower human population density (2.3 hab/km<sup>2</sup>) than Buenos Aires province ([https://www.indec.gob.ar/ftp/cuadros/poblacion/censo2010\\_tomo1.pdf](https://www.indec.gob.ar/ftp/cuadros/poblacion/censo2010_tomo1.pdf)).

### Cloacal samples and bacteria isolates

During 2017-2018, sixty-eight (68) cloacal swabs were sampled: twenty-six (26) from the Kelp Gulls *Larus dominicanus* (Lichtenstein) and twenty-two (22) from the Brown-hooded Gulls *Chroicocephalus maculipennis* (Lichtenstein) from Ensenada, and twenty (20) from Kelp Gulls from Puerto Madryn (Fig. 1). All gulls were sacrificed to carry out other studies under required permits. Every cloacal swab was transported in Cary Blair media (Copan Diagnostics 132C.US®) and kept cool (4°C) until processed. Samples were incubated overnight at 37°C in buffered peptone water. Enriched cultures (30µl) were inoculated on both Mac Conkey agar (Laboratorios Britania S.A. Argentina®) containing 4µg/ml cefotaxime (MC-CTX), and Mac Conkey agar containing 2µg/ml colistin (MC-COL), and incubated for 24 h at 37°C. Colonies with different morphology grown in MC-CTX and MC-COL were subcultured on trypticase soy agar (Laboratorios Britania S.A. Argentina®) for subsequent characterization with conventional phenotypic methods (Koneman et al. 2008).

All scientific bird names were used according to Gill & Donsker (2019).

### Antimicrobial susceptibility testing

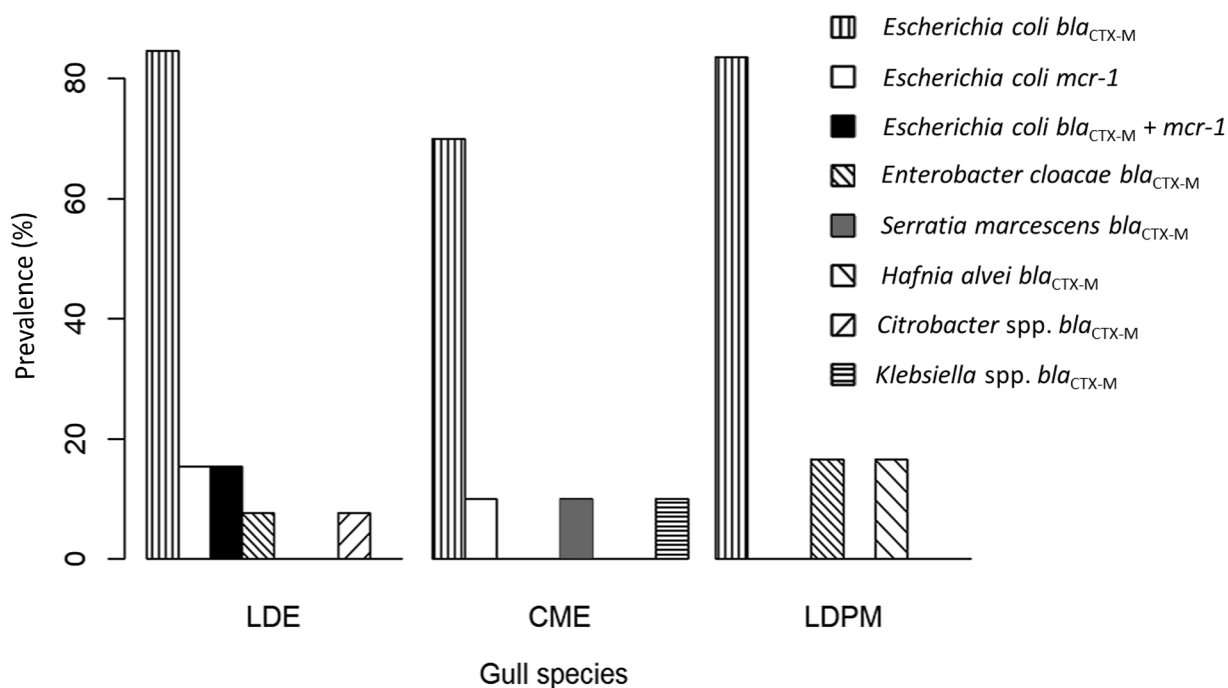
Antimicrobial susceptibility was evaluated by disk diffusion (cefotaxime 30 µg, ceftazidime 30µg, amoxicillin/clavulanic 20/10 µg, imipenem 10µg, ertapenem 10µg (Laboratorios Britania S.A. Argentina®) according to CLSI-M100S 27th ed. guidelines (CLSI 2017) except for colistin where resistance was evaluated as grown or not on screening plates of Müller-Hinton agar (Laboratorios Britania S.A. Argentina®) containing 3µg/ml colistin. *bla*<sub>CTX-M</sub> and *mcr-1* genes were detected by PCR as previously described (Pagani et al. 2003, Liu et al. 2016).

## RESULTS

In Ensenada, 50% (13/26) of the *L. dominicanus* analyzed were positive for *bla*<sub>CTX-M</sub> gene. This one was mainly detected in *E. coli* isolated strains in most of the gulls, 84.6% (11/13). Even more, the 15.4 % (2/13) of the gulls carried the *mcr-1* gene too. Furthermore, the *bla*<sub>CTX-M</sub> gene was detected in *Enterobacter cloacae* and *Citrobacter* spp. strains isolated from two different gull specimens, 7.7% (1/13) respectively (Fig. 2).

From the same locality, 45.4% (10/22) of the *C. maculipennis* analyzed were positive for AMR genes. The 90% (9/10) were positive for *bla*<sub>CTX-M</sub>, mainly harboring by *E. coli* isolated on 7/9 gulls. This gene was also detected from *Klebsiella* spp. on 1/9 gulls, and *Serratia marcescens* on 1/9 gulls. Only one of the 10 *C. maculipennis* positive carried the *mcr-1* gene in *E. coli* isolated strain (Fig. 2).

From Puerto Madryn open landfill, 30% (6/20) of the *L. dominicanus* analyzed, carried *Enterobacteriales* with AMR genes. The *bla*<sub>CTX-M</sub> gene was detected in *E. coli* strains isolated from 5/6 gulls, and in *Hafnia alvei* strain isolated from 1/6 gulls. One of the six gulls carried both *E. coli* and *E. cloacae* strains harboring *bla*<sub>CTX-M</sub>. None of



**Figure 2.** Prevalence (%) of positive gulls for bacteria harboring antimicrobial resistance genes in both studied sites (LDE= *Larus dominicanus* Ensenada, CME= *Chroicocephalus maculipennis* Ensenada, LDPM= *Larus dominicanus* Puerto Madryn).

the gulls from Puerto Madryn were positive for the *mcr-1* gene (Fig. 2).

## DISCUSSION

This study increases the knowledge of seagulls as carriers of *Enterobacteriales* harboring AMR genes in South America. We report two new areas, Buenos Aires and Chubut provinces, as focus of antimicrobial contamination for wild birds; and *C. maculipennis* harboring AMR genes on their *Enterobacteriales* associated for the first time.

The sanitary landfill in Ensenada receives different types of urban, organic and inorganic garbage, which would include poorly recycled pharmaceutical products by the high population density in Buenos Aires province. Also, this landfill is located only a few kilometers from the Río de La Plata, whose shores channel

the sewage of the city. Added to that, the use of antibiotics (colistin and its products) in agricultural and livestock activities that characterize this region may exerts selection pressure on microorganisms, favoring the incorporation and spread of AMR mechanisms, mainly in *C. maculipennis* which is the most abundant gull species who frequents these environments to feed. For all these reasons, resistance mechanisms associated with wild birds were expected to be found in the sanitary landfill from Ensenada city.

In contrast, *mcr-1* gene was not isolated in gulls from Puerto Madryn. This may be related to the number and randomness of the sampled. However, it may be due to biogeographic differences between sampling sites, including population density and types and degrees of anthropogenic activities, which influence environments differently. Those gulls visiting the Municipal bowls from Puerto Madryn are in

contact with other types of garbage. The main discards come from fishing activity, which in this area is handmade; all products come from the sea and do not exposed to antibiotics. Further, population density, agricultural and farming activities are lesser development than in Buenos Aires province, with the consequently reduced use of colistin and its products.

Species detection of *Enterobacterales* harboring resistance genes in gulls that feed on landfills in two different areas from Argentina, contributes to Ramey & Ahlstrom (2020) proposal. These authors highlight that anthropogenic inputs into the environments can act as a focus of infection for gulls and other species, implicating them as indicators of AMR contamination. Although, isolated resistance genes in this study were more frequent in *E. coli*; present results suggest that other species of bacteria (e.g. *E. cloacae*, *Klebsiella* spp., *Citrobacter* spp.) are also playing an important role in the spread of AMR.

The generalist and opportunistic behavior that characterizes gulls and their movements between the breeding and feeding areas become them in potential spreaders of AMR. We agree with Radhouani et al. (2014) that continuing to monitor AMR constitutes a useful tool to evaluate the impact of anthropogenic pressure on environmental health.

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#### Author contributions

Lorenti Eliana: contributed in field work, sampling, and in the conceptualization, methodology and writing of the original draft. Moredo Fabiana: participated in the molecular laboratory methodology and review the draft. Origlia Javier: contributed in sampling process and in the conceptualization of the draft. is Diaz Julia I. contributed in field work and in the conceptualization, supervision, writing, review and edition of the draft. Cremonte Florencia: participated in the writing and review of the draft. Giacoboni Gabriela: contributed in laboratory work and in the conceptualization, methodology, supervision and in the writing of the draft.

