RESEARCH LETTER – Pathogens & Pathogenicity

Audouin’s gull, a potential vehicle of an extended spectrum β-lactamase producing Salmonella Agona

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One Sentence Summary: The genome analysis of multidrug-resistant Salmonella Agona isolated from the migratory seabird Larus audouinii, showing high levels of resistance to public health important antimicrobials, revealed eight resistance genes.

ABSTRACT

The genome of a multidrug-resistant Salmonella Agona isolated from Larus audouinii (Audouin’s gull) in Spain was examined. The isolate showed high levels of resistance to different antimicrobials, including third generation cephalosporins and fluoroquinolones, which is a public health concern as those being used to treat severe salmonellosis in humans. Whole genome sequencing revealed the strain being multilocus sequence type ST13, and eight resistance genes (aadA2, aadB, blaCTX-M-9, blaDHA-1, qnrA1, tetA, sul1 and dfrA16) belonging to seven antimicrobial classes were confirmed, as well as the presence of two plasmids. Migratory Audouin’s gulls have the ability to cover long distances during annual movements. Therefore, they have the potential to disseminate multidrug-resistant Salmonella and resistance genes in the environment and over great geographic distances, contributing to the global dissemination of resistance genes.

Key words: Larus audouinii; Salmonella Agona; multidrug resistance; fluoroquinolones; resistance genes; plasmid

INTRODUCTION

Salmonella enterica serovar Agona was first described in 1961 from cattle in Ghana (Guinee, Kampelmacher and Willems 1961). Since then, Salmonella Agona has increasingly been recognized as a serotype of concern for both, humans and animals, being responsible for several significant food-borne and hospital outbreaks (O’Flanagan et al., 2008). To our knowledge, only a few cases of Salmonella Agona containing extended spectrum β-lactamase (ESBL) genes have been described (Rodriguez et al., 2009). Additionally, there are limited published data relative to the presence of this serotype in wildlife, and none of them have described antimicrobial resistance mechanisms.

Our report describes the presence of a multidrug Salmonella Agona strain isolated from Audouin’s gull (Larus audouinii) in Ebro Delta (northeastern Spain). The aim of this study was to characterize this multidrug-resistant strain using next-generation sequencing. In order to investigate the role of this
seagull species as a potential vehicle in the transmission of antimicrobial resistance, multilocus sequence type (MLST), mechanisms of antimicrobial resistance and the presence of plasmid replicons were determined.

MATERIALS AND METHODS

During the chick-rearing period of 2011, a colony of L. audouinii in the Ebro Delta was sampled. Cloacal swabs (n = 111) were collected and Salmonella isolation was performed using standard culture methods (Antílls et al., 2013). Salmonella serovar was assigned based on the scheme of Kauffmann-White (Grimont and Weill 2007).

Minimum inhibitory concentration (MIC) determination was performed using a commercially prepared, dehydrated panel (Sensititre, TREK Diagnostic Systems Ltd., East Grinstead, England). The following 17 antimicrobials and interpretative criteria for resistance (R) were used: ampicillin, AMP (R ≥ 32 mg L⁻¹); amoxicillin + clavulanic acid, AMC (R ≥ 32 mg L⁻¹); apramycin, APR (R > 16 mg L⁻¹); cefotaxime, CTX (R ≥ 4 mg L⁻¹); cefotaxime, CTX (R > 2 mg L⁻¹); chloramphenicol, CHL (R ≥ 32 mg L⁻¹); ciprofloxacin, CIP (R > 0.064 mg L⁻¹); colistin, COL (R > 2 mg L⁻¹); florfenicol, FFN (R ≥ 16 mg L⁻¹); gentamicin, GEN (R ≥ 2 mg L⁻¹); nalidixic acid, NAL (R ≥ 32 mg L⁻¹); neomycin, NEO (R > 4 mg L⁻¹); spectinomycin, SPT (R > 64 mg L⁻¹); streptomycin, STR (R > 16 mg L⁻¹); sulfamethoxazole, SMX (R ≥ 512 mg L⁻¹); tetracycline, TET (R ≥ 16 mg L⁻¹); and trimethoprim, TMP (R > 16 mg L⁻¹). Interpretation of antimicrobial susceptibility test results was performed by using Clinical and Laboratory Standards Institute (CLSI) clinical breakpoints (CLSI 2006, 2013, 2014) for all antimicrobials except for APR, CIP (decreased susceptibility), COL, FFN, NEO, SPT, STR and XNL, where no values are available to date. The MIC for GEN did not correspond with the detection of the antimicrobial resistance gene aadB applying the CLSI clinical breakpoint for the interpretation why EUCAST epidemiological cut-off values were used. For XNL, CIP (decreased susceptibility), COL, FFN, NEO and STR, EUCAST epidemiological cut-off values were also used according to EUCAST recommendations (http://www.eucast.org). When reporting data using EUCAST epidemiological cut-off values, bacteria should be reported as ‘wild-type’ or ‘non-wild-type’ (Schwarz et al., 2010). Due to the differences in interpretation of the MIC using CLSI/EUCAST and simplicity of the terms, susceptible and resistant has been maintained, even in cases where we are referring to wild-type and non-wild-type strains. Due to the lack of epidemiologic cut-off values in the EUCAST system and CLSI clinical breakpoints, APR and SPT were interpreted according to research results from DTU (DNAmp 2013). Quality control was performed by using reference strain Escherichia coli ATCC 25922 according to CLSI guidelines (CLSI 2006, 2014).

The isolate was sequenced utilizing the Ion Torrent PGM™ (Life Technologies, Paisley, United Kingdom) following the manufacturer’s protocols for 200 bp gDNA fragment library preparation (Ion Xpress™ Plus gDNA and Amplicon Library Preparation), template preparation (Ion OneTouch™ System) and sequencing (Ion PGM™200 sequencing Kit). Raw sequence data have been submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession number ERSS66959. The sequence data were assembled and analysed for MLST, antimicrobial resistance genes and plasmid replicons, using the pipelines available on the Center for Genomic Epidemiology (www.genomicepidemiology.org). Contigs were filtered against the genomes of the reference plasmids harbouring the result-
and subsequently could disseminate it to Ebro Delta colony. Audouin’s gull return to their breeding colonies (Spanish Mediterranean coast, Balearic Islands and Chafarinas Islands) between February and April (http://www.migraciondeaves.org/), where it regularly feeds along the coast. The Ebro Delta site holds 67% of the global population of these seagull species (Gutiérrez and Guinart 2008). The colony feeds largely on fish but also on food discarded by tourists, in marshes, rice fields and occasionally at refuse tips (Mañosa, Oro and Ruiz 2004), which could also be the source of the Salmonella Agona strain or acquisition of resistance genes.

Seagulls are one of the most documented carriers of Salmonella due to their feeding habits (Monaghan et al., 1985; Cizek et al., 1994). Therefore, they can serve as a sentinel for antibiotic pressure from the surrounding farms and urban settings. To better comprehend the overall problem of antibiotic resistance, monitoring wild birds may be a useful parameter for evaluating the impact of anthropic pressure in a specific location. Having into account all control measures taken from the European Union to reduce and minimize the use of antimicrobial resistance to preserve the human treatment of infectious diseases, the contribution of wild life to the emergence of antimicrobial-resistant pathogens will remain uncontrollable. Thus, surveillance programs directed to detect reservoirs of antimicrobial-resistant bacteria in wild birds should be considered.

In conclusion, the presence of Salmonella Agona in seagulls and the transboundary movements of these birds provide them with the potential to spread multidrug-resistant Salmonella beyond its original area. Wild birds, and in this case seagulls, could therefore be considered a risk species. These results emphasize the role of wild birds in the dissemination of multidrug-resistant Salmonella across the world, providing new insight into the impact that the migrations have in the global epidemiology of Salmonella.

SUPPLEMENTARY DATA
Supplementary data is available at FEMSLE online.

ACKNOWLEDGEMENTS
The authors would like to thank Lisbeth Andersen for her technical assistance.
FUNDING
This work was funded by the Center for Genomic Epidemiology (www.genomicepidemiology.org) and by grant FAU2008-00012-C02-01 from INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain). NA is a recipient of a FI fellowship from CUR (DIUE, Generalitat de Catalunya).

Conflict of interest statement. None declared.

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