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CARTAS AL EDITOR

Plasmid-mediated colistin resistance in *Escherichia coli* recovered from healthy poultry

Resistencia a colistina mediada por plásmido en *Escherichia coli* recuperadas de aves de corral sanas

Dear Editor,

Several antibiotics have been massively used at subinhibitory concentrations as growth promoters for more than 6 decades[3] to improve weight gain and therefore, to maximize feed conversion efficiency in animal production. This use is currently under close scrutiny, as it may be – and for sure is – selecting and collaborating for multi-drug resistant enteric bacteria.

As a service to poultry producers we have been monitoring the susceptibility to antimicrobial agents of indicator bacteria isolated from intensive farming systems to support choosing in advance the most effective promoters and to reduce selection pressure. *Escherichia coli* is one of the selected species considered markers for antimicrobial resistance evolution.

From 2013 to date, we have obtained 304 *E. coli* isolates recovered from 129 broiler chicken farms (only poultry growers) located in several provinces of Argentina (Buenos Aires, Córdoba, Entre Rios, Rio Negro and Santa Fe). Isolates were recovered from fresh fecal samples collected randomly from clinically healthy chickens (aged 4–6 weeks). The analysis showed that almost half of them (49%) were found to be resistant to colistin, as determined by microdilution according to EUCAST breakpoint recommendations (version 6.0).

Colistin is considered a last-line antimicrobial agent retaining activity on multiresistant bacteria recovered from humans. Even when resistance was sporadically reported, it was assumed to be obtained by mutation of regulatory genes[4]. However, a gene conferring resistance to colistin was recently reported in conjugative plasmids[2]. This gene (*mcr-1*) was most frequently found in *E. coli* but also in other species such as *Klebsiella pneumoniae* and *Salmonella*[1]. Up to date, a large number of publications demonstrated its presence in isolates collected mainly from animal samples, and to a lesser extent, in samples of human origin.

After selecting approximately one third of our colistin-resistant *E. coli*, all of them were confirmed as *mcr-1* producers by PCR and full gene sequencing. In Argentina, the *mcr-1* determinant was previously reported in *E. coli* clinical strains isolated from inpatients[5].

It should be noted that in our country, many producers have voluntarily stopped using colistin after the technical reports of these (our) results, even before the initial report of plasmid-borne transmission. In this regard, the World Health Organization recommends that the use of colistin be limited for the treatment of clinically affected animals. Moreover, in our country, official regulations governing the administration of these compounds in animal feed have already changed, including a gradual stepwise process with a final goal to completely ban their use by 2019.

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Multiplex PCR assay for genotyping of *Mycobacterium tuberculosis* in Lima, Peru

PCR Múltiple para el Genotipaje de *Mycobacterium tuberculosis* en Lima, Perú

Dear Editor,

In South American countries it is necessary to have molecular methods as epidemiological tools as well as low costs and good level of discrimination capacity for polymorphisms that may enable molecular laboratories to perform the tasks for other regional laboratories having minimum standards. The methodology proposed in this article addresses this need.

We used the Proportion Method[2] for susceptibility testing of *Mycobacterium tuberculosis* isolates against isoniazid (INH), rifampicin (RMP), ethambutol (EMB), para-aminosalicylic acid (PAS), thioacetazone (T), kanamycin (KM) and streptomycin (SM). Cultures were grown on Lowenstein Jensen medium at 37 °C for 21 days, and slides were processed with the Ziehl–Neelsen stain. DNA from samples was extracted by using the phenol chloroform method[6]. In a simple PCR for *M. tuberculosis* strain differentiation, primer Mtb2 (5′-CGGCGGCAACGGCGGCA) was used with primers IS1 (5′-CGGACTCACCGGGGCGGTTCA) and IS2 (5′-CGGACATGCCGGGGCGGTTCA) that anneal at the inverted repeats flanking IS*6110*[7]. PCR was done in a mixture containing 25 pmol of each primer, 1 U of Platinum *Taq* DNA polymerase (Invitrogen), 0.2 mM of each deoxyribonucleotide triphosphate, 10 mM Tris–HCl (pH 8.4), 1.65 mM $MgCl_2$, 50 mM KCl, and 0.1% Triton X-100 and overlaid with mineral oil. Cycling conditions were as follows: denaturation at 94 °C for 5 min, followed by amplification for 35 cycles of 94 °C for 1 min, 62 °C for 1 min, and 72 °C for 1 min, followed by a final extension at 72 °C for 10 min. A total of 20 μl of amplified DNA was subjected to electrophoresis in a 2% agarose gel, detected by ethidium bromide staining, and visualized under UV light. For the genetic polymorphism study, we used the Bionumerics program version 5.0 (Applied-Maths).

The drug resistance study showed that out of 49 strains belonging to TB patients in the Guillermo Almenara Irigoyen National Hospital, 18 strains (36.74%) were drug-sensitive, 10 (20.4%) were drug-resistant TB (DR) and 21 (42.8%) were multidrug-resistant TB (MDR), 3 of which were TB/HIV cases. Forty-two (42) different banding patterns were observed, which were classified into 10 clusters (Fig. 1). We suspect that cases MT009 and MT029 belong to heteroresistant strains, i.e. mixed wild-type and mutant strains because the banding pattern seems to overlap[5].

The transmission study showed Cluster II. Two male patients with MDR-TB, one of whom (MT014) had undergone previous MDR-TB treatment, were both hospitalized; Cluster IV. Two male patients with TB/HIV co-infection, both residing in the same district; Cluster VI. Two male patients with TB/HIV co-infection, both living in the same district and receiving their treatment in the same hospital, one of them (MT048) with DR TB and the other (MT054) with a sensitive case; Cluster VIII. Three male patients aged 44, 34 and 28 years, respectively, two of whom were brothers (MT033 and MT041) and the other a neighbor (MT032), all of them sensitive cases. The other clusters did not have an epidemiological link. A statistical risk study was performed[4,13] and the result was that the patients with HIV infections had the highest contagion risk in our population ($p = 0.174$; OR = 3.150; CI = 0.568–17.477). The repetition rate was good (Cronbach's alpha = 0.82). This genotyping method could be an alternative for other PCR-based typing procedures, such as spoligotyping and MIRU-VNTR typing as cited in other studies[7] and could help in the study of transmission relationship with heteroresistance, HIV-TB patients and outbreaks. Our TB survey system has many complications[3,9,11] and the lack of surveillance in DOTS[3] results in patients having a great diversity of genotypes and drug-resistant profiles[1,5], as well as heteroresistance of wild type to resistant, resistant to resistant, and wild type to MDR strains[1,4,8,10,12,13]. Our country needs a strategy based on epidemiology with molecular tools that will assist us in the analysis of the genetic diversity existing in Peru.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.