

INTEGRON-RELATED ANTIMICROBIAL RESISTANCE IN NOSOCOMIAL AND COMMUNITY ACQUIRED INFECTIONS

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HTVC

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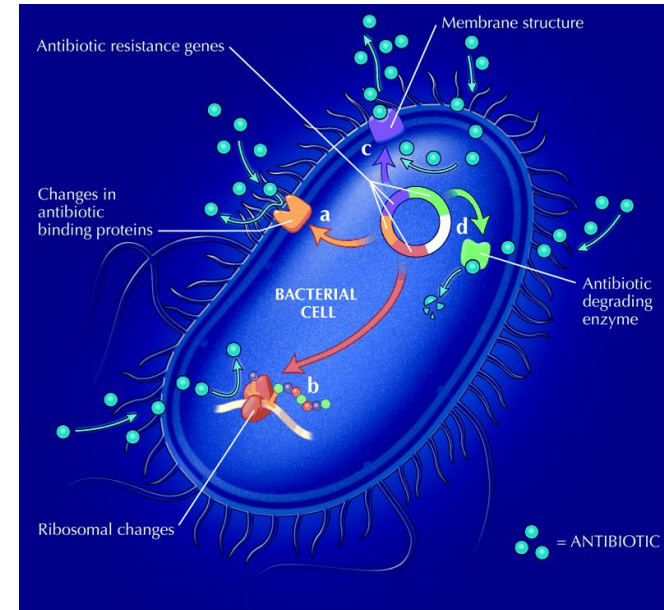


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OBJECTIVES

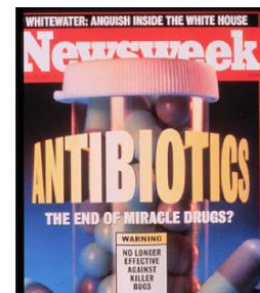


- The focus of interest of our group is the study of different microbiological issues related to antimicrobial resistance in gram-negative bacteria in our geographical area.

ANTIBIOTIC RESISTANCE: AN EMERGING PROBLEM

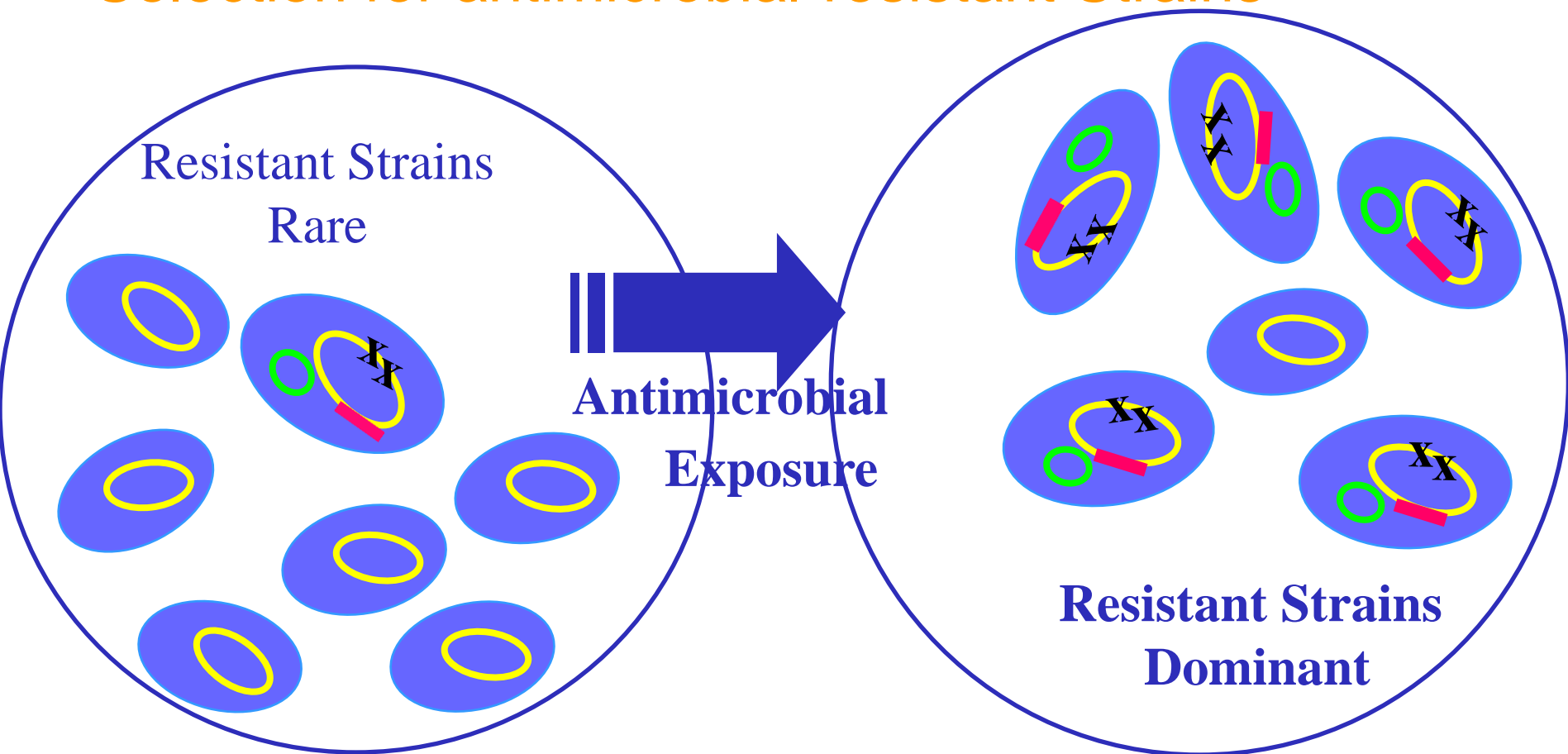
80 years latter

1928



- One of the most pressing causes of health concern from individual (clinical), ecological (public health) and social perspective, aggravated by the lack of new antimicrobials development.

Selection for antimicrobial-resistant Strains



Emergence of antibiotic resistance is an inevitable consequence of antimicrobials use in any setting (humans, animals, agriculture).



12 Steps to Prevent Antimicrobial Resistance: Hospitalized Adults

- 12 Break the chain
- 11 Isolate the pathogen
- 10 Stop treatment when cured
- 9 Know when to say “no” to vanco
- 8 Treat infection, not colonization
- 7 Treat infection, not contamination
- 6 **Use local data**
- 5 Practice antimicrobial control
- 4 Access the experts
- 3 Target the pathogen
- 2 Get the catheters out
- 1 Vaccinate

Prevent Transmission

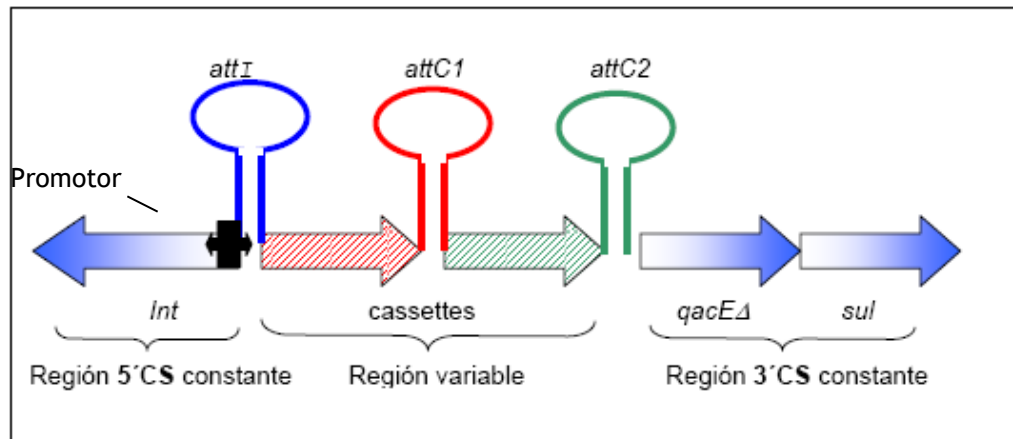
Use Antimicrobials Wisely

Diagnose & Treat Effectively
Prevent Infections

- Characterization of mechanisms involved in antimicrobial resistance in gram-negative bacteria.
 - Particularly resistance to β -lactams (**amoxicillin-clavulanate**) and **Integron-associated resistance**.
 - Optimization of antibiotic therapy: improvement in effectiveness and prevention of emergence of resistances.
- Molecular epidemiology studies of isolates displaying troublesome or uncommon resistance mechanisms aimed at establishing the reasons for their spread and designing strategies to bring it under control
- Horizontal transfer of genetic resistance elements (antibiotic policy)
- Spread of a limited number of clones (infection control measures)

INTEGRONS

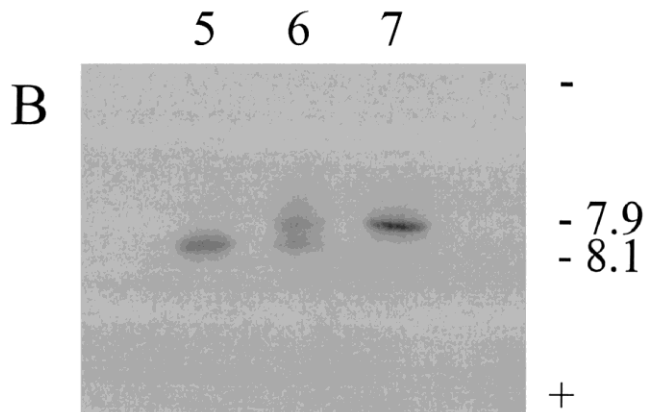
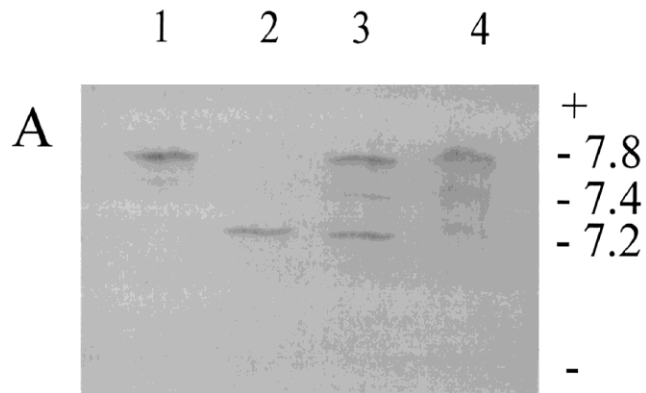
- Integrons
 - widespread, potentially mobile, rather small, genetic elements.
 - generally located on plasmids or transposons.
 - able to incorporate and mobilize resistance gene cassettes throughout a site-specific recombination system directed by an integrase.
- Class 1 integrons are the commonest.
- Two highly conserved regions (5'CS and 3'CS ends) flanking a variable region where gene cassettes encoding resistance to a wide range of antibiotics are inserted.



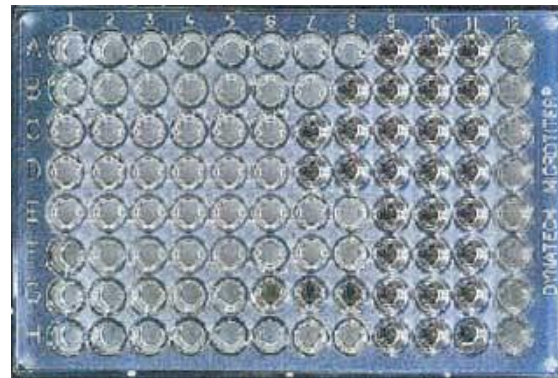
- Important and efficient (low biological cost) source for the acquisition, and spread of resistance and cross-resistance in gram-negative bacteria.

PROJECTS (I)

- Characterization of mechanisms involved in resistance to associations of B-lactams plus β -lactamase inhibitors in *Escherichia coli* isolates recovered in Terres de l'Ebre



METHODS: Isoelectrophocusing (characterization of β -lactamases according to pI) and **spectrophotometry** (β -lactamase specific activity and IC_{50} of clavulanate)



Caracterización de la resistencia a asociaciones de betalactámicos e inhibidores de betalactamasas en *Escherichia coli*. Influencia del tipo y nivel de producción de betalactamasas

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FUNDAMENTO: Se pretende caracterizar la resistencia de *Escherichia coli* en nuestro medio a cuatro asociaciones de betalactámicos e inhibidores de betalactamasas (β Lac), investigando la influencia del tipo y nivel de producción de β Lac.

MÉTODOS: Determinamos la concentración mínima inhibitoria (CMI) a ampicilina/sulbactam (A/S), amoxicilina/clavulánico (A/C), ticarcilina/clavulánico (T/C) y piperacilina/tazobactam (P/T) de 245 cepas de *E. coli* resistentes a ampicilina, aisladas consecutivamente en nuestro laboratorio entre septiembre de 1995 y marzo de 1996. Las β Lac producidas por estos aislados fueron identificadas por isoelectroenfoque (IEF) y cuantificadas espectrofotométricamente.

RESULTADOS: La sensibilidad a A/S, A/C, T/C y P/T fue del 9,4%, 86,9%, 64,5% y 89,4%, respectivamente. Las cepas con una sola β Lac que coenfocaba con TEM-1 fueron las mayoritarias (215/245), seguidas por aquellas que producían una enzima coenfocando con SHV-1 (7/245). Encontramos una correlación significativa entre la actividad β Lac de las 215 cepas TEM-1 y su CMI a A/S ($r = 0,53$; $p < 0,001$), A/C ($r = 0,46$; $p < 0,001$) T/C ($r = 0,58$; $p < 0,001$) y P/T ($r = 0,42$; $p < 0,001$). La comparación entre la actividad enzimática de los aislados pertenecientes a distintas categorías de susceptibilidad evidenció diferencias significativas ($p < 0,05$) para las

The characterization of resistance to β -lactamics and β -lactamase inhibitors in *Escherichia coli*. The influence of the type level of β -lactamase production

BACKGROUND: The aim of this study was to characterize resistance of *Escherichia coli* in our environment to four associations of β -lactams and β -lactamase inhibitors (β Lac) studying the influence of the type and level of β Lac production.

METHODS: The minimum inhibitory concentration (MIC) to ampicillin/sulbactam (A/S), amoxycillin/clavulanic acid (A/C), ticarcillin/clavulanic acid (T/C) and piperacillin/tazobactam (P/T) assessed was in 245 strains of *E. coli* resistant to ampicillin, consecutively isolated in our laboratory from September 1995 to March 1996. The β Lac produced by these isolates were identified by isoelectrofocusing and spectrophotometrically quantified. **RESULTS:** The sensitivity to A/S, A/C, T/C and P/T was of 9.4, 86.9, 64.5 and 89.4%, respectively. The strains with only one β Lac which cofocused with TEM-1 were the most frequent (215/245), followed by those producing a cofocusing enzyme with SHV-1 (7/245). A significant correlation was observed between β Lact activity of the 215 TEM-1 strains and their MIC at A/S ($r = 0.53$; $p < 0.001$), A/C ($r = 0.46$; $p < 0.001$), T/TC ($r = 0.58$; $p < 0.001$), and P/T ($r = 0.42$; $p < 0.001$). The comparison

Mechanisms of reduced susceptibility to amoxicillin–clavulanic acid in *Escherichia coli* strains from the health region of Tortosa (Catalonia, Spain)

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ABSTRACT

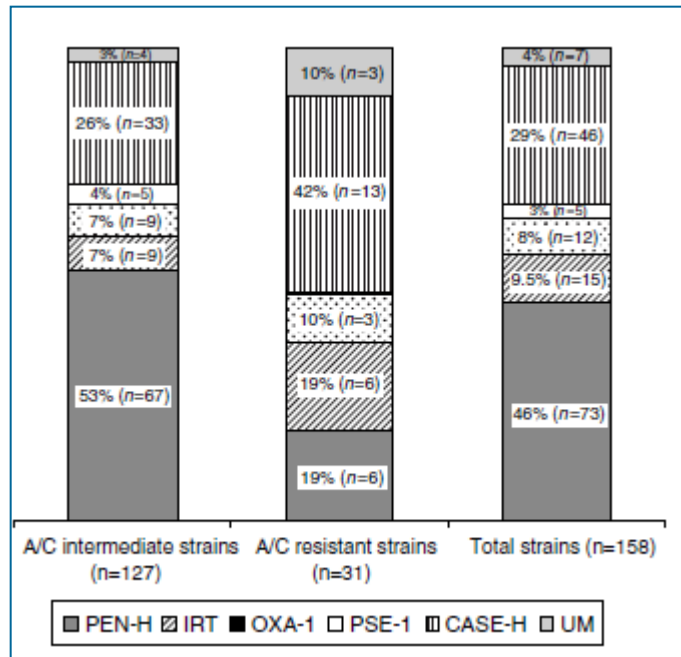
This study investigated the mechanisms involved in reduced susceptibility to amoxicillin–clavulanic acid and the prevalence of enzymes compatible with inhibitor-resistant TEM (IRT) β -lactamases produced by *Escherichia coli* isolates from patients in north-eastern Spain. The resistance mechanisms of 158 strains showing resistance or intermediate resistance to amoxicillin–clavulanic acid among 1122 ampicillin-resistant clinical isolates of *E. coli* were assessed on the basis of their β -lactam resistance phenotypes. β -Lactamases produced by strains showing resistant phenotypes suggestive of inhibitor-resistant penicillinase production were characterised by their isoelectric point. Specific activity and the concentration of clavulanic acid required to inhibit β -lactamase activity by 50% (IC₅₀) were determined in strains harbouring enzymes that focused at pI 5.2 or 5.4 in order to achieve presumptive identification of IRT β -lactamases. Resistance phenotypes were consistent with overproduction of TEM-1, TEM-2 or SHV-1 β -lactamases in 56 strains, with AmpC cephalosporinase hyperproduction in 46 strains, and with production of inhibitor-resistant penicillinases in 49 strains. Of the latter isolates, 17 produced moderately high or high levels of enzymes co-focusing with TEM-1, 17 produced enzymes co-focusing with OXA-1 ($n = 12$) or with PSE-1 ($n = 5$), either alone or in association with TEM-1, while only 15 produced enzymes with a phenotype characteristic of IRT β -lactamases. It was concluded that resistance to amoxicillin–clavulanic acid in *E. coli* isolates from this area was mainly associated with presumptive overproduction of TEM-1, TEM-2 or SHV-1 β -lactamases (46%) or of AmpC cephalosporinase (29%), while the occurrence of enzymes categorised as IRT β -lactamases was unusual (9.5%).

Keywords *Escherichia coli*, amoxicillin–clavulanic acid resistance mechanisms, IRT β -lactamases

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RESULTS (I)

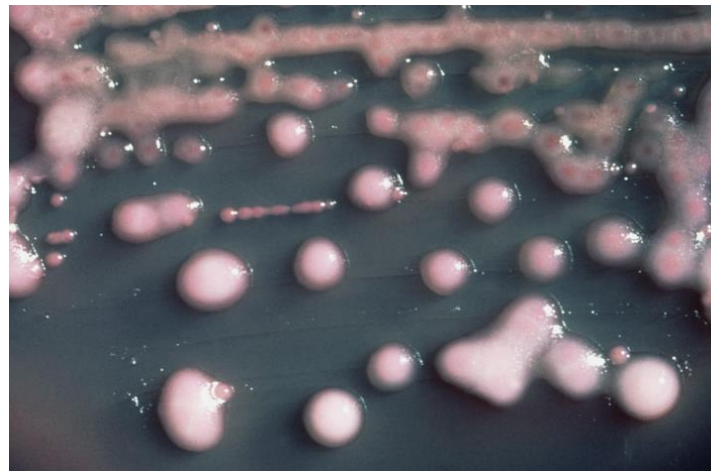


- A number of mechanisms responsible for resistance to amoxicillin-clavulanate in *Escherichia coli*
 - TEM-1 hyperproduction
 - AMPc hyperproduction or plasmidic cephamycinses.
 - IRT β -lactamases production
 - OXA-1 production

- Only one previous surveillance study in Spain to know the prevalence of IRT enzymes in *E. coli* (specific resistance mechanism against β -lactamase inhibitors).
 - Mechanism difficult to recognize by conventional antibiogram.
 - Inclusion of low concentrations of cefepime in microdilution panels is a helpful tool for distinguishing OXA-1 from IRT β -lactamases.
 - Low but on the increase prevalence specially in community acquired UTI (related to the wide use of amoxicillin-clavulanate?).

Molecular epidemiology and characterization of resistance mechanisms involved in reduced susceptibility to amoxicillin-clavulanate in *Klebsiella pneumoniae* isolates from a chronic-care center.

Striking rate of decreased susceptibility to amoxicillin-clavulanate among cephalosporin-susceptible *K. pneumoniae* isolates recovered from patients from a chronic-care hospital, where amoxicillin-clavulanate was the most prescribed antibiotic followed by fluorquinolones, since 2003 to 2009.



- **PCR amplification or PCR-mapping assays and nucleotide sequencing:**
 - tracking of genes encoding β -lactamases and other antibiotic resistance determinants,
 - chromosomal mutations in *gyrA* and *parC*
 - presence of class 1 integrons and identification of resistance cassettes within the integron variable region.
- **ERIC-PCR:** DNA fingerprinting to assess epidemiological relationship between isolates.
- **Conjugation experiments:** To ascertain the eventual location of resistance genetic elements in conjugative plasmids.



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Title: Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin-clavulanate in *Klebsiella pneumoniae* isolates from a chronic-care center.

Article Type: Original Article

Keywords: *Klebsiella pneumoniae*; amoxicillin-clavulanate resistance; IRT-11; OXA-1; class 1 integrons.

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Abstract: The aim of this work was to investigate the molecular epidemiology and mechanisms responsible for reduced susceptibility to amoxicillin-clavulanate among cefazolin-susceptible *Klebsiella pneumoniae* isolates from patients admitted to a chronic-care institution. Fifty-one of the 171 (29.8%) *K. pneumoniae* isolates recovered between 2006 and 2008 were non-susceptible to amoxicillin-clavulanate. Forty-five of them were susceptible to cefazolin and nucleotide sequencing analysis revealed that 19 produced IRT-11 and the remaining 26 were OXA-1 producers. All the former isolates harboured the *aac(6')-1b-cr-blaOXA-1* cassette array, which in 23 isolates was located together with *catB3* and *arr3* within a class 1 integron and associated with *qnrS2* (in three cases the integron lacked the *qacEΔ1* and *sul1* or *sul3* genes). Genotyping analysis carried out by ERIC-PCR identified three different patterns among IRT-11 producing isolates (E1 to E3) with E1 being the most prevalent (63%), while the OXA-1 producing isolates were assigned to patterns E3 and E3a (isolates carrying typical class 1 integrons), E4 (isolates carrying defective integrons) and E5 (isolates without integrons). Genes encoding IRT-11 and OXA-1 were transferred by conjugation and *aac(6')-1b-cr* and *qnrS2* were systematically co-transferred with *blaOXA-1*. Our results demonstrate that the high prevalence of decreased susceptibility to amoxicillin-clavulanate among *K. pneumoniae* isolates from a chronic-care hospital was mainly due to the simultaneous spread of two different clones, one of which comprised isolates that produced IRT-11 and the other one isolates that had acquired either the *blaOXA-1* gene located in a class 1 integron and linked to *qnrS2* or the *blaIRT-11* gene.

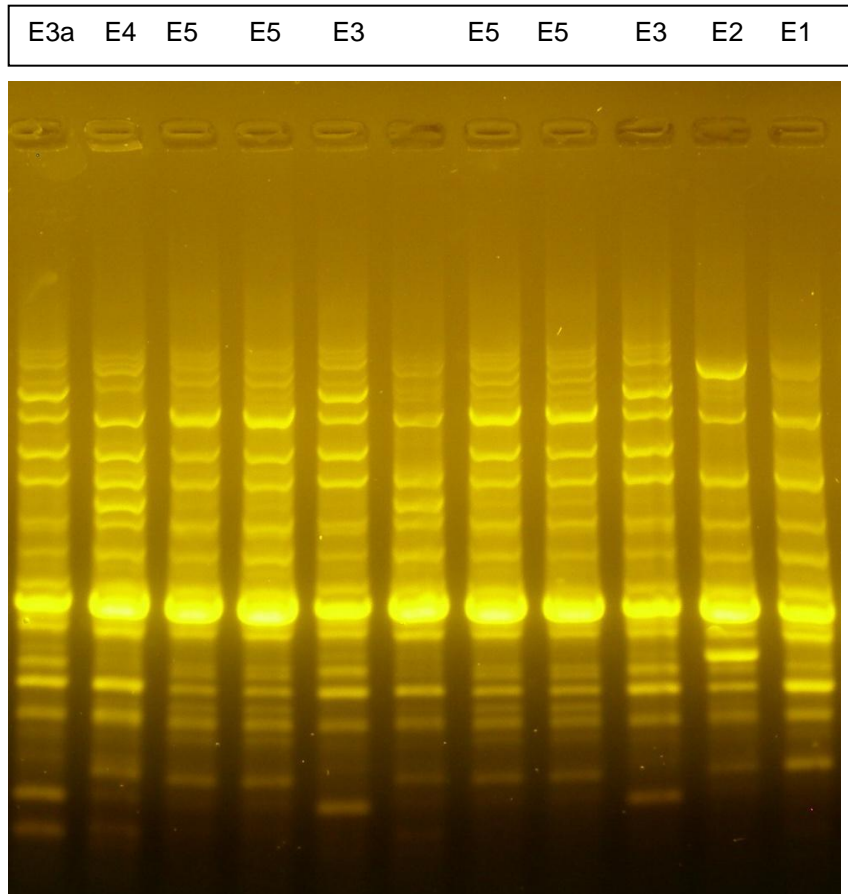
RESULTS (II)

- **Mechanisms involved:** Coexistence of isolates displaying two different β -lactamases, quite uncommon in this species, conferring specific resistance to β -lactamase inhibitors (IRT-11 and OXA-1 β -lactamase).
- In almost all cases gene encoding OXA-1 located within a class 1 integron together with other antibiotic resistance determinants (aminoglycosides, quinolones, chloramphenicol and rifampicin) and associated to *qnrS2* (transferible resistance to quinolones) in a conjugative plasmid (Estructure only reported in an aquatic isolate of *Aeromonas allosacharophila*).



- An identical integron, although in a different genetic environment - associated to genes encoding plasmidic cephamycinases - has been described in some sporadic human Enterobacterial isolates.
- Three isolates carried an atypical class 1 integron lacking *qacEΔ1* and *sul* (typical of environmental isolates).

ERIC-PCR Patterns



Lanes 1-5 and 7-8: OXA-1 producing isolates
Lanes 9-11: IRT-11 producing isolates
Lane 6: amoxicillin/clavulanate-susceptible isolate

- IRT-11 producing isolates ascribed to three clones (E1 to E3) → Clonal dissemination and horizontal transfer.
- OXA-1 producing isolates carrying typical class integron ascribed to two closely related clones (E3 and E3a) → Clonal dissemination.
- OXA-1 producing isolates carrying defective class 1 integron ascribed to one clone (E4) → Clonal dissemination.

But in 2009 two *qnrS2 Escherichia coli* isolates harbouring the same atypical integron.

CONCLUSIONS (II)

- Second description reporting an outbreak involving IRT enzymes (the first one occurred in a chronic-care facility in France).
- Importance of diversifying antibiotics used in an institution in order to avoid selective pressure which favours emergence of resistances.
 - High consumption of ACL in the hospital lead to the emergence and selection of a resistance mechanism typical of community-acquired isolates.
- Outstanding: adherence to standard infection control precautions.
 - Cross-infection between patients lead to dissemination of resistant clones.
- Role played by integrons in co-selection (use of a single antimicrobial may select for resistance to other antibiotics) and horizontal transfer of resistance.

Role of integrons in antibiotic resistance in a typical zoonotic community-acquired infection: Food-borne disease caused by Non-typhoidal *Salmonella*.



■ Class 1 integron dissemination among multiresistant *Salmonella enterica* human isolates from Terres de l'Ebre

ORIGINALES

Integrones de clase 1 en aislados de *Salmonella enterica* productores de diferentes tipos de betalactamasas recogidos en la región sanitaria de Tortosa

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OBJETIVO. Estudiar la frecuencia de integrones de clase 1 en aislados de *Salmonella enterica* productores de diferentes tipos de betalactamasas recogidos en la región sanitaria de Tortosa e intentar determinar los genes de resistencia que llevan insertados.

MÉTODOS. Se investigó mediante reacción en cadena de la polimerasa (PCR) la presencia de integrones de clase 1 y de los genes *aadA1*, *aadA2*, *dfrA1*, *tem-1*, *oxa-1* y *pse-1* en la región variable de éstos, en 100 aislados de *S. enterica* (30 *S. enteritidis*, 56 *S. ser* Typhimurium y 14 de otros serotipos) resistentes a ampicilina recuperados consecutivamente en nuestro laboratorio entre 2000 y 2001. Las betalactamasas se caracterizaron

Class 1 integrons among *Salmonella enterica* isolates producing different types of beta-lactamases from the health region of Tortosa (Spain)

OBJECTIVE. To assess the frequency of class 1 integrons among isolates of *Salmonella enterica* producing different types of beta-lactamases from the health region of Tortosa, and to attempt to establish the resistance genes located within their variable regions.

METHODS. The presence of class 1 integrons and of *aadA1*, *aadA2*, *dfrA1*, *tem-1*, *oxa-1* and *pse-1* resistance genes within their variable regions was investigated by PCR

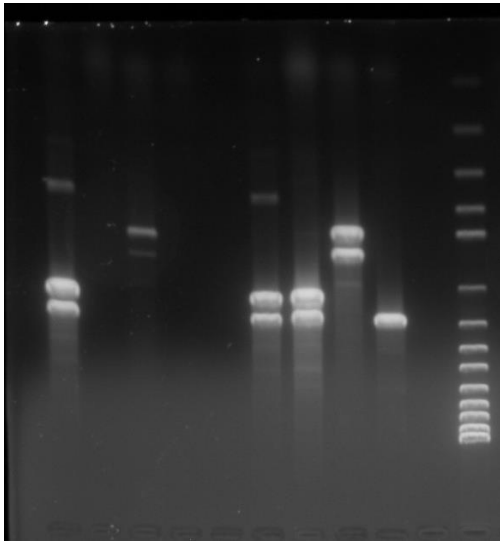
- **Molecular epidemiology of infections caused by multiresistant integron-carrying human *Salmonella enterica* serotype Typhimurium isolates from the health Region of Terres de l'Ebre**

Multidrug resistance related to class 1 integrons in human *Salmonella enterica* serotype Typhimurium isolates and emergence of atypical *sul3*-associated integrons

Sir,

Salmonella enterica serotype Typhimurium is one of the leading causes of food-borne disease in developed countries. Multidrug resistance, which is generally linked to the presence of integrons and is often related to antibiotic use in food animals, has been increasingly reported in this serovar within the last 20 years [1]. Integrons are versatile genetic elements that play an important role in the dissemination of multidrug-resistant (MDR) Gram-negative microorganisms and in the co-selection of resistance. Most integrons found in clinical isolates of *Salmonella* spp., as in other Enterobacteriaceae, belong to class 1 and possess the *sul1* sulphonamide resistance gene in the 3' conserved segment (3'CS)

RESULTS AND CONCLUSIONS (III)

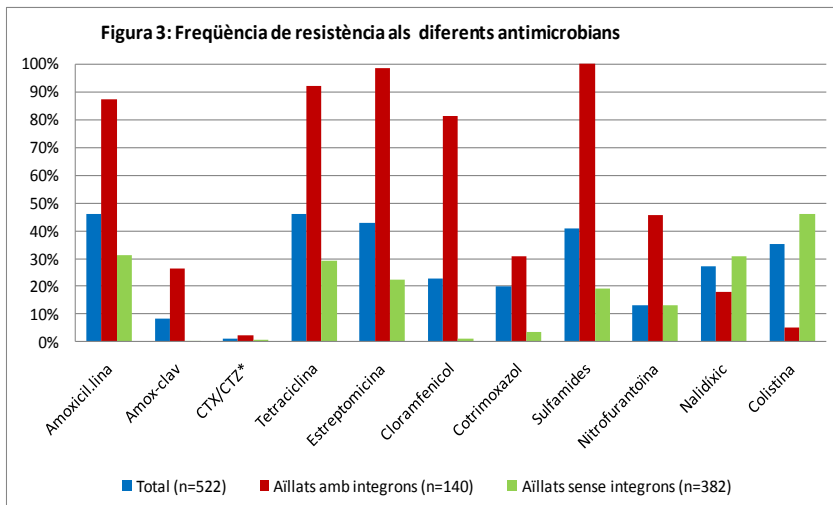


- *dfrA1- aadA1*
- *dfrA12-orf-aadA2*
- *dfrA12-orf-aadA2-cmlA1-aadA1*
- *bla_{oxa-1} -aadA1*
- *aadA2/ bla_{pse-1}*

- Multiresistance linked to integrons is widespread among human *Salmonella enterica* isolates from our geographical region
 - 59% of MR isolates carried integrons.
 - 10 different types of integrons in 14 different serotypes of *Salmonella*.

- Resistance genes born by or linked to integrons usually confer resistance to antibiotics employed in livestock and veterinary medicine but also to some that are the first choice in treatment of systemic human salmonellosis (amoxicillin-clavulanate, broad-range cephalosporins).

- Need of wise antibiotic use in animals: selective pressure posed by antibiotic use in food animals may have negative impact on the treatment of human infections.



RESULTS AND CONCLUSIONS (III)

- Dramatic increase in the prevalence of serotype Typhimurium, usually more resistant and in some cases more virulent, to the detriment of serotype Enteritidis.
 - Efforts to minimize the transmission of all food-borne pathogens by insistence on good hygiene practices on farms, in abattoirs, during distribution and marketing of food and in food preparation.
- **First description of**
 - The presence of integrons in some unusual serotypes (Kapemba, Goettingen and *S. Sub. I* [9,12: lv:-])
 - The emergence of *S. enterica* isolates harbouring atypical *sul3*-associated integrons, not previously reported in Spain, that are not detected by conventional methods.
 - The emergence of *S. Typhimurium* isolates possessing a particular plasmid-borne integron (2 Kb/ *bla*_{oxa-1} -*aadA1*) which nowadays have become endemic in Spain and have displaced the pandemic SGI1 isolates.
 - Most isolates of both types belonged to a predominant clone and shared the same resistance phenotype except for resistance to nitrofurantoin (related with fraudulent use of nitrofurans in animal husbandry?).

- Going on with surveillance of local antimicrobial resistance and underlying mechanisms and epidemiology
 - Follow of resistance trends
 - Alert for new resistance mechanisms
 - Identify risk factors for antimicrobial resistance
 - Design and evaluate interventions to control antimicrobial resistance
- Extending the scope of our research on resistance mechanisms
 - Transferable quinolone resistance
 - Characterization of an SHV-derived β -lactamase conferring an atypical resistance phenotype.



THE TEAM



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Moltes gràcies

