Universitat Internacional de Catalunya
Facultat de Medicina

Tesis doctoral

CANVIS A L'EPIDEMIOLOGIA DE Klebsiella pneumoniae PRODUCTORA DE β-LACTAMASA D'ESPECTRE ESTÈS

Mariona Xercavins i Valls

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DIRECTORA

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Línia de recerca

MALALTIES INFECCIOSES: PREVENCIÓ, DIAGNÒSTIC I TRACTAMENT

uic barcelona

Recordant els pares,
per la Núria i l'Enric i
pel Jaume.

No es pot separar la música de la vida.
Pau Casals

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A l'arribar al final d'aquest llarg camí, i a la meva edat qui m'ho hauria de dir, voldria agrair a un seguit de persones que m'ha ajudat moltíssim i que han estat al meu costat en el curs d'aquesta tesi.

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Que tinguem sort...

RESUM

Klebsiella pneumoniae productora de β-lactamasa d'espectre estès (KpBLEE) s'ha associat tradicionalment amb infeccions relacionades amb l'assistència sanitària i amb brots hospitalaris.

Aquest microorganisme ha experimentat un gran canvi en el tipus de β-lactamasa que vehiculitza: fins al final de la dècada dels 90 les soques KpBLEE eren portadores dels enzims SHV i TEM i eren, principalment, d'origen hospitalari i a l'inici del segle XXI emergeixen els enzims CTX-M que provoquen que l'epidemiologia variï ràpidament i es constata l'enzim CTX-M-15 com el més prevalent.

Els brots nosocomials per KpBLEE productora deCTX-M-15 presenten unes característiques diferents dels causats per soques productores de SHV o TEM: es distribueixen àmpliament per les diferents unitats d'un hospital, la mortalitat és més baixa i són freqüentment policlonals.

A la nostra epidemiologia local ens va sorprendre que alguns pacients amb infeccions per KpBLEE diagnosticades a l'hospital eren definides com a comunitàries per l'absència de factors de risc associats amb l'atenció sanitària.

Probablement, existeixin reservoris d'aquest patogen fora de l'hospital. Sabem que existeix una continuïtat entre l'àmbit hospitalari i el comunitari, de tal manera que els microorganismes poden procedir d'un d'aquests àmbits i disseminar-se a l'altre.

Per tant, la **hipòtesi** del treball és que KpBLEE ha passat de ser un patogen exclusivament nosocomial a ser un patogen que també es troba a la comunitat, especialment a soques productores de CTX-M-15.

L'estudi s'ha dut a terme a l'Hospital Universitari Mútua Terrassa, Barcelona, hospital amb 400 llits amb una mitjana anual de 97.524 estades i els seus 9 centres d'assistència primària (CAPs) que atenen a una població d'uns 350.000 habitants.

En el **primer article** s'han estudiat les infeccions del tracte urinari per KpBLEE diagnosticades als CAPs en el període 2010-2014.

La prevalença de les ITU d'origen comunitari (ITU-Co) per KpBLEE va augmentar d'un 2,4% al 2010 al 10,3% el 2014 (p=0,01), mentre que les causades per *K. pneumoniae* no productora de BLEE van restar estables (7,5% el 2010 i 8,5% el 2014; P=0.08).

Els factors de risc associats a ITU-Co degudes a KpBLEE van ser l'exposició a les cefalosporines i el ser resident d'un centre sociosanitari o residència.

Quasi 2/3 dels casos van ser d'origen estrictament comunitari.

Els enzims CTX-M-15 van ser els més freqüents.

En el **segon article** es va realitzar un estudi prospectiu de cohorts durant l'any 2015 a l'Hospital Universitari Mútua de Terrassa. Es van incloure tots els pacients adults nous consecutius amb aïllament de KpBLEE a qualsevol mostra clínica obtinguda per pràctica de rutina clínica. Els pacients amb antecedents d'infecció / colonització prèvia per KpBLEE i els pacients ingressats a la Unitat de Cures Intensives van ser exclosos .

Es van identificar 60 casos consecutius i no es va detectar cap cas de transmissió creuada.

Les infeccions o colonitzacions es van classificar com a adquirides a l'hospital (52%), relacionades amb l'assistència (40%) i adquirides a la comunitat (8%).

Es va detectar una elevada diversitat genètica. Mitjançant rep-PCR les 60 soques es van classificar en 36 patrons i per MLST es van identificar 16 *seqüenciotips.*

En el 96,7% de les soques la β-lactamasa d'espectre estès va ser CTX-M-15.

Els dos estudis presentats han permès aclarir part de l'epidemiologia de la KpBLEE al nostre àmbit. Per una banda hem identificat infeccions d'origen veritablement comunitari i per l'altra hem constatat una gran diversitat genètica entre les soques aïllades a nivell nosocomial sense poder demostrar transmissió creuada.

Aquestes dades ens podran ajudar, tant, a la racionalització de les polítiques de control d'infecció durant l'ingrés hospitalari, valorant en quins pacients amb KpBLEE i a quines àrees de l'hospital caldria realitzar precaucions de contacte o seria suficient amb precaucions estàndard, així com també ajudar a realitzar el disseny dels tractaments empírics en funció dels factors de risc de KpBLEE per tal d'optimitzar l'ús d'antibiòtics.

ABSTRACT

ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) has frequently been associated with healthcare-related infections and with hospital outbreaks.

This microorganism has experienced a great change in the class of β-lactamase it harbourers. Until the end of 1990s the strains of (ESBL-Kp) almost always due to SHV and TEM types and were mainly hospital-acquired. At the beginning of the 21st century with the emergency of the CTX-M enzymes, the epidemiology had to change quickly so as to consider CTX-M-15 enzyme as the more prevalent.

The nosocomial outbreaks caused by ESBL-Kp CTX-M-15 are somewhat different from previous SHV or TEM outbreaks: they are widely distributed in hospitals wards, the mortality seems to be lower and are frequently polyclonal.

In our local epidemiology we were surprised that some patients with infections by ESBL-Kp diagnosed in the hospital were defined as community-acquired by the absence of risk factors associated with healthcare.

Probably there are reservoirs of this pathogen outside the hospital.

We know that there is a continuity between the hospital setting and the community, in such a way that the microorganisms can come from one of these settings and spread to the other.

Therefore, the **hypothesis** of work is that ESBL-Kp has ceased to be a pathogen exclusively nosocomial to be a pathogen that is also found in the community, especially in the strains that produce CTX-M-15.

This study was carried out in the Hospital Universitari Mútua de Terrassa, a 400-bed care hospital with an annual mean number of 97,524 stays and nine primary care centres for attending a population of 350,000 inhabitants.

In the first **article**, we studied community-onset urinary tract infections (CO-UTIs) due to ESBL-Kp diagnosed during 2010-2014.

The prevalence of CO-UTIs due to ESBL-Kp increased from 2,4% in 2010 to 10,3% in 2014 (P=0.01), whilst the prevalence of CO-UTIs due to non ESBL-Kp remained stable (7,5% in 2010 to 8,5% in 2014; P=0.08).

Being a nursing home resident and previous cephalosporin use were risk factors independently associated with CO-UTI due ESBL-Kp.

Almost two-thirds of the case patients their infection can be considered truly community-acquired.

CTX-M-15 was the most common type of ESBL identified.

Second **article:** in 2015 a prospective cohort study was conducted at Hospital Universitari Mútua Terrassa. All new consecutive adult patients with ESBL-Kp isolates from any specimens obtained by routine clinical practice were included. Patients with previous known infection/colonization by ESBL-Kp were excluded, as were adult patients admitted to intensive care units.

Sixty consecutive index cases were identified. No cases of cross-transmission were found.

The infection/colonization were classified as hospital-acquired (52%), healthcare-related (40%) and community-acquired (8%).

High clonal diversity was detected. The isolates were classified into 36 patterns (rep-PCR) and in 16 sequence types.

The enzyme CTX-M-15 were detected in 96,7% of the isolates.

The two studies presented have helped us better understand the epidemiology of KpBLEE in our area. We have identified truly community-origin infections and we have found great genetic diversity among isolated nosocomial strains without being able to demonstrate cross-transmission.

These data may be useful to rationalize infection control policies during hospital admission, assessing in which patients with KpBLEE and in which areas of the hospital contact precautions should be performed or would be sufficient with standard precautions, as well as helping to design empirical treatments based on KpBLEE risk factors in order to optimize the use of antibiotics.

ÍNDEX

RELACIÓ D'ABREVIATURES

ADN: Àcid desoxiribonucleic

AP-PCR: amplificació arbitrària de fragments genètics

BLEE: β-lactamasa d'espectre estès

CAPs: Centres d'assistència primària

CLAV: àcid clavulànic

CMI: Concentració Mínima Inhibitòria

EcnoBLEE: *Escherichia coli* no productora de β-lactamasa d'espectre estès

ECP: Electroforesi camp polsat

EDTA: àcid etilediaminatetraacètic

EUCAST: European Committee on Antimicrobial Susceptibility Testing

IC: Interval de Confiança

ITU: Infecció del tracte urinari

ITU-Co: Infecció del tracte urinari d'origen comunitari

KpBLEE: *Klebsiella pneumoniae* productora de β-lactamasa d'espectre estès

KpnoBLEE: *Klebsiella pneumoniae* no productora de β-lactamasa d'espectre estès

mL: mililitre

MLST: Multilocus sequence typing

MMR: Microorganismes multiresistents

NA: No aplica

NI: No inclòs

OMS: Organització Mundial de la Salut

OR: Odds ratio

PBP: Penicillin binding proteins

PCR: Reacció en Cadena de la Polimerasa

Rep: seqüències repetitives

ST: *seqüenciotip*

STROBE: Strengthening the reporting of observational studies in epidemiology

UCI: Unitat de cures intensives

ufc: unitats formadores de colònies

I. INTRODUCCIÓ

1. TAXONOMIA

Klebsiella pneumoniae taxonòmicament pertany a l'ordre d' Enterobacterales i a la família de les Enterobacteriaceae.

El gènere *Klebsiella* deu el seu nom al patòleg alemany Edwin Klebs (1834-1913) que va descriure la presència de bacteris a la via aèria de pacients morts per pneumònia (1875), d'aquí el nom de *Klebsiella pneumoniae*, malgrat no queda clar que fos veritablement *Klebsiella* sinó *Streptococcus pneumoniae.*

2. IMPORTÀNCIA CLÍNICA

K. pneumoniae té un paper important com a agent etiològic de malalties infeccioses, sent el segon enterobacteri més freqüentment relacionat amb infeccions nosocomials i comunitàries, després d' *Escherichia coli.*

És un patogen primari que pot causar infeccions del tracte urinari, bacterièmies, abscessos hepàtics, infeccions del tracte biliar, peritonitis, infeccions de ferides quirúrgiques, pneumònia, meningitis, etc. [1].

3. ENTEROBACTERIS: MECANISMES DE RESISTÈNCIA ALS β-LACTÀMICS:

La resistència als antibiòtics β-lactàmics, segons el mecanisme d'actuació, pot ser deguda a 4 mecanismes de resistència diferents:

3.1 Alteracions a les proteïnes fixadores de penicil·lina (*penicillin binding proteins,* PBPs):

Les PBPs són enzims que catalitzen la síntesi de peptidoglicà i també són la diana dels antibiòtics β-lactàmics i es produeixen per mutacions puntuals.

Les modificacions a la seva estructura confereixen una pèrdua d'afinitat de les PBPs pels antibiòtics β-lactàmics i la hiperproducció de les PBPs pot donar lloc a una disminució de la sensibilitat del bacteri per aquest tipus d'antibiòtics. Aquest mecanisme està descrit tant a microorganismes grampositius com a gramnegatius, però no s'ha objectivat a *Klebsiella pneumoniae.*

3.2 Modificació a la permeabilitat de la membrana externa:

Aquest fenomen és degut bàsicament a mutacions en els gens que codifiquen o regulen l'expressió de les proteïnes que conformen les porines (canals de membrana). La resistència als antibiòtics β-lactàmics pot produir-se per pèrdua de les porines, reducció del seu nombre o per una modificació de les mateixes. Amb freqüència aquest mecanisme s'associa a altres mecanismes de resistència, com la producció de β-lactamases o la presència de bombes d'expulsió activa. A *K. pneumoniae* s'han identificat dues porines principals: OmpK35 i OmpK36 [2,3].

3.3 Bombes d'expulsió activa:

Es produeixen mitjançant l'adquisició de gens que codifiquen o permeten l'expressió de bombes d'expulsió o d'altres existents es modifiquen per a poder expulsar determinades molècules antimicrobianes.

Les bombes d'expulsió activa AcrAB i OqxAB s'han detectat freqüentment *K. pneumoniae* [4,5].

3.4 Producció de β-lactamases:

Són enzims produïts pel propi bacteri. En els enterobacteris és el mecanisme més important de resistència al grup d'antibiòtics β-lactàmics.

3.4.1 Definició:

Les β-lactamases són enzims capaços d'inactivar els antibiòtics β-lactàmics (penicil·lines, cefalosporines, monobactàmics i carbapenèmics). Són hidrolases que trenquen l'enllaç amida de l'anell β-lactàmic i produeixen derivats àcids sense propietats bactericides, fet que evita que aquests antibiòtics es puguin unir a les proteïnes diana (*penicillin-binding proteins,* PBP), impedint així la seva acció sobre la formació de la paret bacteriana i, per tant, la lisi bacteriana.

El nivell de resistència depèn directament del grau d'afinitat de l'antibiòtic, de les propietats hidrolítiques de cada enzim i del nivell de producció d'aquest.

Dels diferents mecanismes de resistència als β-lactàmics, la producció de β-lactamases és la causa principal de la resistència dels enterobacteris a aquest grup d'antibiòtics [6].

3.4.2 Classificació de les β-lactamases:

La gran quantitat d'enzims i de perfils d'hidròlisi ha comportat diferents tipus de classificacions de les β-lactamases. Les més utilitzades són la basada amb l'estructura molecular d'Ambler [7] i la funcional de Bush, Medeiros i Jacoby [8].

La classificació d'Ambler (1980) es basa en l'estructura molecular i la seqüència d'aminoàcids de les β-lactamases. Reconeix 4 tipus moleculars (A,B,C i D). Els tipus A, B i D tenen una serina en el seu centre actiu, i les del grup B una o més molècules de zinc (metal·lo-enzims). **Taula 1.**

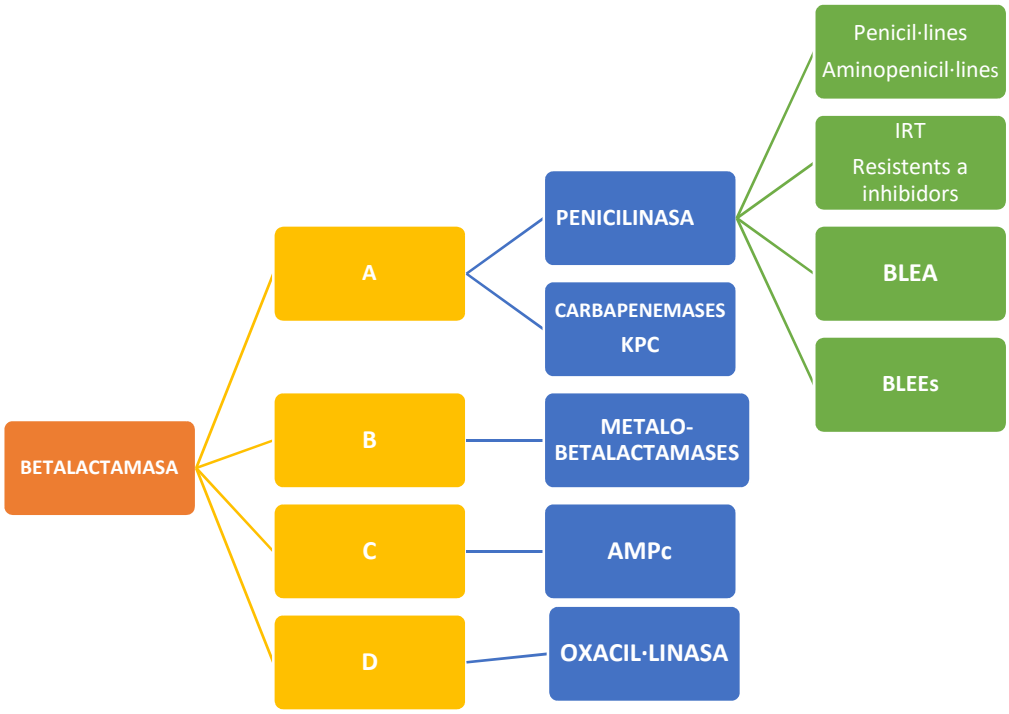
La classificació de Bush, Mediros i Jacoby (1995) es basa en els substrats que hidrolitzen els enzims i en la inhibició de la seva activitat per diferents components: àcid clavulànic, EDTA, aztreonam i oxacil·lina. En aquesta classificació es defineixen 4 grups funcionals integrant propietats bioquímiques, d'estructura molecular i la seqüència de nucleòtids. **Taula 2.**

Segons l'esquema de Bush, Jacoby i Medeiros, el grup 2 és el més abundant i en ell s'inclouen moltes β-lactamases de gran importància clínica.

Les seves característiques són:

- S'agrupen les β-lactamases que pertanyen a les classes A i D d'Ambler.
- Presenten un residu de serina en el seu centre actiu
- La majoria s'inhibeixen per àcid clavulànic
- La localització dels gens $_{bla}$ és plasmídica en la majoria dels casos.

Taula 1. Classificació de les β-lactamases segons Ambler



Taula 2. Classificació de les β-lactamases segons Bush, Jacoby i Medeiros

Grup Busb, Jacoby i Medeiros	Classe Ambler	Substrat preferit	Inhibides per		Enzims representatius
			CLAV	EDTA	
1	C	Penicil·lines, cefalosporines d'espectre restringit i estès, cefamicines i monobactans	-	-	CMY-2 a 13, LAT-1, MOX-1 i 2, FOX 1 a 6, ACT-1, MIR-1, DHA-1 i 2, ACC-1, CFE-1, alguns enzims cromosòmics de bacteris gramnegatius
2a	A	Penicil·lines	+	-	Penicil·linases de bacteris grampositius
2b	A	Penicil·lines i cefalosporines de 1ª generació	+	*	TEM-1, TEM-2, SHV-1
2be	A	Penicil·lines, cefalosporines de 1ª, 2ª i 3ª generació i monobactans	+	-	Nombroses variants d' SHV- i TEM, CTX-M, PER, VEB, GES-1, IBC-1
2br	A	Penicil·lines i cefalosporines de 1ª, 2ª i 3ª generació	-	-	TEM-50 (CMT-1), TEM-68 (CMT-2), TEM-89 (CMT-3)
2c	A	Penicil·lines i carbenicil·lina	+	-	PSE-1, PSE-3 a 5
2d	D	.Penicil·lines i cloxacil·lina .Penicil·lines, cloxacil·lina, β-lactàmics d'espectre estès, monobactans i cefalosporines de 3ª generació . Penicil·lines, oxacil·lina i carbapenèmics	+/- +/- +	- - -	Nombroses variants d'OXA Algunes derivades d'OXA-2 i OXA-10, OXA-18, 29, 30,31,32 i 45. OXA-23 a 27, 40, 48, 54
2e	A	Penicil·lines i cefalosporines de 1ª, 2ª i 3ª generació	+	-	B-lactamasa cromosòmica de *B. fragilis, B. uniformis, B. vulgatus, C. diversus, P. mirabilis, Y. enterocolitica, , C. koseri* i *C. sediaki*
2f	A	Penicil·lines, cefalosporines de 1ª a 4ª generació i carbapenèmics	+	-	NMC-A, SME-1 a 3, IMI-1, KPC-1 a 3, GES-2
3	B	Penicil·lines, cefalosporines de 1ª a 4ª generació i carbapenèmics	-	+	IMP-1 a 13, VIM 1 a 7, SPM-1, NDM
4	NI	Penicil·lines	-	-	Enzim cromosòmic de *Burkholderia cepacia*

NI: no inclòs / CLAV: àcid clavulànic / EDTA: àcid etilediaminotetraacètic

4. Klebsiella pneumoniae PRODUCTORA DE β-LACTAMASA D'ESPECTRE ESTÈS

És el microorganisme objecte de l'estudi. *Klebsiella pneumoniae* és intrínsecament resistent a ampicil·lina i carbenicil·lina per la producció de β-lactamasa tipus SHV-1 cromosòmica [9]. Fins al començament dels anys 80 el tractament estàndard d'elecció en infeccions greus produïdes per aquest bacteri eren les cefalosporines de 3ª generació, però això canviarà amb l'aparició de les β-lactamases d'espectre estès (BLEEs).

Les BLEEs són enzims produïts per bacils gramnegatius que pertanyen al grup 2 de Bush, Jacoby i Medeiros [8] i es caracteritzen per hidrolitzar les cefalosporines d'ampli espectre i els monobactams, però no les cefamicines ni els carbapenèmics, a més a més, es caracteritzen per ser inhibides per l'àcid clavulànic. Estan codificades generalment per plasmidis, afavorint-se així la seva disseminació i amb freqüència presenten coresistència a altres antibacterians com aminoglucòsids, cotrimoxazol i quinolones [10].

Les BLEEs van ser inicialment descrites a Alemanya i França a l'inici de la dècada del 1980 i actualment estan descrites arreu del mon [11]. Les primeres BLEEs identificades deriven dels enzims TEM-1, TEM-2 i SHV-1 i presenten una o més substitucions a la seqüència d'aminoàcids, fet que els confereix capacitat per a remodelar el centre actiu de l'enzim, i són capaces d'hidrolitzar llavors a cefalosporines de tercera i quarta generació.

Les β-lactamases del grup CTX-M, que a l'actualitat són el grup predominant, van ser identificades al final d'aquesta dècada dels anys 80 del segle XX [11,12].

Les CTX-M deuen el seu nom a la seva potent activitat hidrolítica sobre la cefotaxima i el seu origen és totalment diferent de de les TEM i SHV. Les CTX-M van ser adquirides de soques ambientals de *Kluyvera* spp. [13].

Des de la descripció inicial de les CTX-M aquestes β-lactamases s'han disseminat epidèmicament entre els enterobacteris i actualment són el tipus predominant [14,15]. Un factor important d'aquest predomini és l'àmplia disseminació de clones productores de CTX-M. De fet, la disseminació clonal de CTX-M-15 a *E. coli* del grup filogenètic B2 i *seqüenciotip* (ST) 131 ha estat identificat com el més prevalent en molts països [16].

Actualment estan registrades més de 220 variants de CTX-M [17]. Es divideixen en 5 subfamílies (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 i CTX-M-25, [18]) i aquestes gairebé desplacen les altres BLEEs, incloent les variants de TEM i SHV.

Amb l'aparició de les CTX-M l'epidemiologia de les BLEEs ha canviat radicalment i, per exemple, la majoria d'aïllaments d' *E. coli* BLEE són d'origen comunitari [19]. Actualment, CTX-M és la β-lactamasa d'espectre estès més comú a *E. coli*: CTX-M-15 és la més freqüent [20], seguida de CTX-M-14, la qual es troba fonamentalment al Sud-est asiàtic [21]. Estudis recents mostren que CTX-M-27 està emergint a certes parts del món, especialment Japó [22] i Europa [23].

Fins el final de la dècada del 1990 les soques de *Klebsiella pneumoniae* productora de BLEE (KpBLEE) eren principalment adquirides a l'hospital i eren del tipus SHV i TEM. Amb l'inici del segle XXI també s'ha observat el fenomen ocorregut a *E. coli* i la majoria de BLEEs són CTX-M i concretament CTX-M-15 [20]. La prevalença d'aquest enzim ha augmentat significativament i és la BLEE més freqüentment descrita a moltes parts del món [15]. La ràpida emergència d'*E. coli* productor de CTX-M-15 ha generat un gran reservori genètic del qual altres espècies, com *K. pneumoniae*, han pogut adquirir el gen de resistència [24].

Per altra banda, aquesta disseminació mundial de KpBLEE productora de CTX-M-15 és el resultat tant de la disseminació de clones epidèmiques com de diferents plasmidis que confereixen també altres mecanismes de resistències, fenòmens que contribueixen a la seva selecció i que estan implicats en la disseminació intercontinental de les CTX-M [25].

4.1 Canvis epidemiològics:

KpBLEE ha sigut responsable d'un gran nombre de brots hospitalaris. Tradicionalment, descrits a Unitats de Cures Intensives, a on el consum antibiòtic és més alt i la potencial transmissió pacient a pacient més probable [26]. A la **taula 3** es mostra un recull dels brots hospitalaris per KpBLEE.

Aquests brots s'associen amb un increment de la mortalitat, un augment de l'estada hospitalària i del cost sanitari [27].

Per altra banda, KpBLEE pot actuar com a important reservori de gens associats a resistència antibiòtica i transferir-los a altres microorganismes mitjançant transferència plasmídica intergenèrica [28].

Aquest microorganisme ha experimentat un gran canvi en el tipus de β-lactamasa que vehiculitza. Els primers brots descrits de KpBLEE eren produïts per β-lactamases tipus SHV [29] i TEM [30] i es limitaven a àrees restringides de l'hospital (sobretot unitats de cures intensives neonatals i pediàtriques).

A partir de l'any 2.000 es descriuen els primers brots de KpBLEE per enzims CTX-M, específicament CTX-M-15 [20,31], fet que provoca que l'epidemiologia variï ràpidament.

Des de que ha emergit aquest enzim CTX-M-15 els brots nosocomials presenten unes característiques diferents dels causats per SHV o TEM: es distribueixen àmpliament per les diferents unitats d'un hospital general (plantes convencionals d'hospitalització) més que limitar-se a una sola unitat, són freqüentment policlonals i la mortalitat, quan es reporta, sembla més baixa que la descrita dels brots per SHV i TEM [26]. De fet, l'alta diversitat clonal ha sigut reportada en situacions de brot [32] i de no brot [33].

També es descriuen brots deguts a diferents enterobacteris identificats simultàniament però portadors del mateix enzim. En aquests casos rarament s'identifica la font del brot [30,34].

Per una altra banda, la interfase entre hospitals i comunitat pot quedar desdibuixada, i aquest fenomen que ja era evident a *E. coli* ara també s'observa a *K. pneumoniae*. A la **Taula 4** es mostren diferents estudis que avalen aquestes dades en portadors fecals, mostres clíniques comunitàries, aigua, menjar, animals de companyia.

Taula 3. Brots hospitalaris

Estudi, any	País	Tipus enzims	Clonalitat MLST	Pacients totals (n)	Pacients infectats (n)	Localització	Font transmissió	Mortalitat (%)	Ref.
Cotton *et al*, 2000	Sud-àfrica	No descrit	Clonal	159	32	UCI Neonatal	Paneroles	8	[35]
Parasakthi *et al*, 2000	Malàisia	No descrit	Policlonal	8	5	Oncologia pediàtrica	Sabó líquid, flascons dispensadors	40	[36]
Rebuck *et al*, 2000	EUA	No descrit	Policlonal	23	23	UCI Pediàtrica	Desconeguda	20	[37]
Fiett *et al*, 2000	Polònia	TEM-68, TEM-47	Policlonal	22	NA	Pediatria	Desconeguda	NA	[38]
Macrae *et al*, 2001	Regne Unit	SHV-2	Clonal	22	NA	UCI Neonatal	Pacient índex, transmissió per mans	13	[39]
Silva *et al*, 2001	Mèxic	SHV-5	Clonal	31	21	UCI Neonatal	Transmissió per mans	63	[29]
González-Vertiz *et al*, 2001	Mèxic	No descrit	Clonal	23	NA	UCI Neonatal	Desconeguda	NA	[40]
Komatsu *et al*, 2001	Japó	MEN-1	Clonal	23	18	UCI i plantes hospitalització	Desconeguda	12,5	[41]
Quale *et al*, 2002	EUA	SHV-5 principalment	Policlonal	281	NA	15 hospitals regionals	Desconeguda	NA	[42]
Pessoa-Silva *et al*, 2003	Brasil	Tipus SHV	Clonal	207	13	UCI Neonatal	Ambient. Llet. Transmissió creuada	NA	[43]

Estudi, any	País	Tipus enzims	Clonalitat MLST	Pacients totals (n)	Pacients infectats (n)	Localització	Font transmissió	Mortalitat (%)	Ref.
Ayan *et al*, 2003	Turquia	No descrit	Policlonal	33	NA	UCI Neonatal	Ambient. Transmissió creuada	75	[44]
Gruteke *et al*, 2003	Holanda	SHV-5	Clonal	24	17	Medicina Interna, UCI cirurgia	Pacients colonitzats. Transmissió creuada.	5	[45]
Brenwald *et al*, 2003	Regne Unit	Tipus CTX-M	Clonal	36	NA	Pacients ingressats i ambulatoris	Desconeguda	NA	[46]
Ben-Haumouda *et al*, 2003	Tunísia	No descrit	Policlonal	40	21	Unitat Neonatal	Desconeguda	NA	[47]
Duarte *et al*, 2003	Portugal	GES-1	Clonal	30	NA	Unitats mèdiques, quirúrgiques i UCI	Desconeguda	NA	[48]
Cartelle *et al,* 2004	Espanya	No descrit	Clonal	21	17	UCI Neonatal	Cas índex i ambient	NA	[49]
Gupta *et al*, 2004	EUA	No descrit	Clonal	19	9	UCI Neonatal	Ungles artificials, estetoscopi	NA	[50]
Miranda *et al,* 2004	Mèxic	SHV-5	Policlonal	184	NA	Hospital pediàtric	Disseminació clonal	NA	[51]
Bouallège-Godet *et al*, 2005	Tunísia	No descrit	Policlonal	57	22	UCI Neonatal	Termòmetres rectals, transmissió per mans	NA	[52]
Moodley *et al,* 2005	Sud-àfrica	No descrit	Clonal probablement	NA	26	UCI Neonatal	Preparació endovenosa de múltiple dosi	85	[53]

Estudi, any	País	Tipus enzims	Clonalitat MLST	Pacients totals (n)	Pacients infectats (n)	Localització	Font transmissió	Mortalitat (%)	Ref.
Van't Veen *et al,* 2005	Holanda	No descrit	Clonal	NA	7	UCI	Camilles	57	[54]
Mamlouk *et al,* 2006	Tunísia	CTX-M-15, CTX-M-16	Policlonal	27	NA	Diferents unitats hospital general	Desconeguda	NA	[34]
Martins *et al,* 2006	Brasil	No descrit	NM	55	6	Unitat trasplantament renal	Desconeguda	NA	[55]
Cassettari *et al,* 2006	Brasil	No descrit	Policlonal	36	7	UCI Neonatal	Onicomicosi personal sanitari	NA	[56]
Mena *et al,* 2006	Espanya	CTX-M-1	NA	NA	51	UCI	Desconeguda	NA	[57]
Mazzariol *et al*, 2007	Holanda	SHV-31	Clonal	85	NA	UCI	Desconeguda	NA	[58]
Laurent *et al*, 2008	Bèlgica	CTX-M-15	Clonal	30	9	UCI	Desconeguda	26	[59]
De Oliveira *et al*, 2008	Brasil	CTX-M-12, CTX-M-59, SHV-5	Policlonal	49	NA	UCI Neonatal	Llet materna	NA	[60]
Abdel-Hady *et al,*2008	Egipte	SHV-2, SHV-1	NA	380	27	UCI Neonatal	Ambient. Transmissió creuada	28	[61]
Ko *et al,* 2008	Corea	CTX-M-14, SHV-5, SHV-11, SHV-12, SHV-14	ST 2	43	NA	Diferents unitats hospital general	Desconeguda	NA	[62]
Velasco *et al*, 2009	Espanya	TEM-4	Dues clones	161	13	UCI Neonatal	Desconeguda	7	[30]

Estudi, any	País	Tipus enzims	Clonalitat MLST	Pacients totals (n)	Pacients infectats (n)	Localització	Font transmissió	Mortalitat (%)	Ref.
Lytsy *et al,* 2008	Suècia	CTX-M-15	Clonal	64	NA	Diferents unitats hospital general	Desconeguda	NA	[63]
Carrër *et al*, 2009	França	CTX-M-15	Clonal 36 *K. pneumoniae,* 21 *E. coli*, 10 *E. cloacae*	36	3	UCI	Desconeguda	NA	[64]
Randrianirina *et al,* 2009	Madagascar	CTX-M-15, SHV-2	Clonal	2	10	Unitats pediàtriques	Aigua aixeta i tubs aspiració	30	[65]
Vranic-Ladavac *et al*, 2010	Croàcia	CTX-M-15	Policlonal	NA	162	Diferents unitats hospital general	Desconeguda	NA	[66]
Aumeran *et al,* 2010	França	CTX-M-15	Clonal	16	12	Unitat endoscòpia	Endoscopi	NA	[67]
Dumpis *et al,* 2010	Letònia	CTX-M-15	ST 199	NA	32	Diferents unitats hospital general i UCI	Desconeguda	NA	[68]
Dedeic-Ljubovic *et al,* 2010	Bòsnia-Herzegovina	CTX-M-15	Policlonal	NA	35	Diferents unitats hospital general	Desconeguda	NA	[69]
Calbo *et al,* 2011	Espanya	CTX-M-15	Clonal	156	35	Diferents unitats hospital general	Alimentària, manipuladors d'aliments i cuina com a reservori	14	[31]
Webster *et al,* 2011	Regne Unit	CTX-M-15	ST 490	NA	28	4 hospitals àrea Oxford	Desconeguda	NA	[70]

Estudi, any	País	Tipus enzims	Clonalitat MLST	Pacients totals (n)	Pacients infectats (n)	Localització	Font transmissió	Mortalitat (%)	Ref.
Damjanova *et al,* 2011	Hongria	CTX-M-15, SHV-2	ST 274	NA	27	5 centres de salut	Desconeguda	NA	[71]
Guyot *et al,* 2012	França	CTX-M-15	Policlonal	NA	23	UCI Neonatal	Cas índex i transmissió creuada	NA	[72]
Lin *et al,* 2012	Xina	No descrit	Policlonal	65	28	UCI Neonatal	Colonització personal sanitari i superfícies de contacte	2,6	[73]
Rettedal *et al,* 2013	Noruega	CTX-M-15	Clonal	65	NA	UCI Neonatal	Desconeguda	0	[74]
Valsdottir *et al,* 2017	Islàndia	CTX-M-15	ST-336	2.478	69	Servei Rehabilitació	Desconeguda	NA	[75]
Lenglet *et al,* 2018	Haití	No descrit	ST-37	55	22	UCI Neonatal	Desconeguda	NA	[76]
Boonstra *et al,*2020	Holanda	CTX-M-15, SHV-28	ST-307	14	11	Servei Rehabilitació	Rentadora	NA	[77]

NA: No aplica

Taula 4. Comunitària

Estudi, any	País	Tipus mostra	Nombre mostres estudiades	% KpBLEE	TIPUS ENZIM	REF.
Mesa *et al*, 2006	Espanya	Recerca portadors fecals comunitaris	948	0,1	No descrit	[78]
Prado *et al,* 2007	Brasil	Aigües residuals hospitalàries	20	100	No descrit	[79]
Kader *et al*, 2009	Aràbia Saudí	Recerca portadors fecals comunitaris	716	4,4	No descrit	[80]
Randrianirina *et al,2009*	Madagascar	Aigua aixeta	40	NA	CTX-M-15, SHV2	[65]
Andriatahina *et al*, 2010	Madagascar	Recerca portadors fecals pediàtrics a l'ingrés	244	9,8	No descrit	[81]
Sasaki *et al*, 2010	Tailàndia	Recerca portadors fecals comunitaris	160	3,1	CTX-M	[82]
Herindrainy *et al*, 2011	Madagascar	Recerca portadors fecals comunitaris	484	2,9	CTX-M-15 (92,8%)	[83]
Dolejska *et al,* 2011	República Txeca	Aigües residuals municipals	68	5,9	CTX-M-15	[84]
Calbo *et al,* 2011	Espanya	Recerca portadors fecals i mostres clíniques	1.100	14,2	CTX-M-15	[31]
Rahman *et al,* 2011	Egipte	Recerca portadors fecals comunitaris	632	24	No descrit	[85]
Ruppé *et al,* 2012	França	Recerca portadors fecals a l'ingrés	500	1	No descrit	[86]
Isendhal *et al*, 2012	Guinea-Bissau	Recerca portadors fecals nens >5 anys a urgències	408	22,3	CTX-M-1 grup (93,4%)	[87]
Lonchel *et al,* 2012	Camerún	Recerca portadors fecals comunitaris	208	1	CTX-M-15	[88]
Luvsansharav *et al*, 2012	Tailàndia	Recerca portadors fecals comunitaris	417	4,7	CTX-M	[89]
Poirel *et al,* 2013	França	Recerca portadors fecals animals de companyia	110	13,6	CTX-M-15	[90]
Donati *et al,* 2014	Itàlia	Mostres clíniques gossos i gats	1.984	0,7	CTX-M-15, SHV-28	[91]
Bonnedahl *et al,* 2014	Alaska	Mostres fecals gavines	55	63,6	CTX-M-15, SHV-12, SHV-102	[92]

Estudi, any	País	Tipus mostra	Nombre mostres estudiades	% KpBLEE	TIPUS ENZIM	REF.
Ewers *et al,* 2014	Alemanya i altres 15 països europeus	Mostres clíniques animals de companyia i cavalls	1.519	8	CTX-M-15	[93]
Pons *et al*, 2015	Moçambic	Mostres clíniques (origen comunitari)	449	4,2	CTX-M-15 dominant	[94]
Arana *et al,* 2017	Espanya	Urocultius comunitaris: 2007-10 2011-14	30.778	 1,6 5,5	No descrit	[95]
Maharjan *et al,* 2018	Nepal	Recerca portadors fecals comunitaris	510	6,67	TEM, CTX-M, SHV	[96]
Abayneh *et al,* 2018	Etiòpia	Urocultius comunitaris	342	1,2	No descrit	[97]
Fatima *et al,* 2018	Índia	Urocultius positius comunitaris	247	3,6	No descrit	[98]
Atterby *et al*, 2018	Cambodja	Recerca portadors fecals comunitaris	307	1,6	SHV	[99]
Mahamat *et al* 2019	Txad	Recerca portadors fecals comunitaris	100	5	CTX-M-15 (80%)	[100]
Priyadharshana *et al,* 2019	Sri-Lanka	Urocultius comunitaris	405	1	CTX-M (75%)	[101]

NA: No aplica

4.2 Detecció fenotípica de les BLEEs:

La detecció ràpida de la resistència als antimicrobians així com la seva caracterització és una prioritat en els laboratoris de microbiologia clínica.

La detecció de les BLEE en el laboratori no sempre és fàcil, ja que depèn de la seva expressió fenotípica, i aquesta ve condicionada per la quantitat d'enzim produït pel bacteri. La seva detecció es basa en la capacitat d'aquests enzims d'hidrolitzar les cefalosporines de tercera i quarta generació i els monobactams, disminuint per tant la sensibilitat del bacteri a aquests antibacterians i que es posa de manifest en un increment de les concentracions mínimes inhibitòries (CMI) o en una disminució dels halos d'inhibició quan es realitza la tècnica de difusió amb discs. Una altra de les característiques d'aquests enzims és que són inhibides per l'àcid clavulànic i que no presenten activitat hidrolítica en front de la cefoxitina [102].

La majoria de proves fenotípiques desenvolupades per a la detecció de BLEE es basen en l'activitat inhibitòria de l'àcid clavulànic [102]:

- Tècnica de sinèrgia amb doble disc en la que la presència de la BLEE es detecta tant per la resistència o disminució dels diàmetres d'inhibició d'alguns o tots els substrats com per l'efecte sinèrgic produït entre les cefalosporines de tercera o quarta generació o els monobactans i l'àcid clavulànic.
- Una altra variant és la utilització de discs de cefalosporines combinats amb àcid clavulànic: es valora un increment del diàmetre inhibitori de la cefalosporina de 3ª en presència d'àcid clavulànic de ≥ 5 mm respecte al de la cefalosporina corresponent sense àcid clavulànic.
- Tècnica de difusió en gradient (E-test) amb tires combinades de cefalosporines amb i sense inhibidor. Una disminució de la CMI ≥ 3 dilucions amb la presència d'àcid clavulànic confirma la producció de BLEE.

- Sistemes automatitzats: diferents sistemes automatitzats incorporen detecció de BLEE: Vitek 2® (bioMérieux), MicroScan WallAway ®(Beckman Coulter), BD Phoenix® (Becton Dickinson).

4.3 Detecció genotípica de les BLEEs:

Per a la confirmació genotípica de la presència de BLEEs existeixen des de diferents tipus de PCR (reacció en cadena de la polimerasa) a seqüenciació massiva.

El mètode basat amb la detecció de DNA-microarray és el recomanat per l'EUCAST [103] i és el que hem emprat en els nostres estudis. Aquesta metodologia consisteix en la detecció, mitjançant una anàlisi d'imatges, de la hibridació d'una molècula diana a una sonda específica immobilitzada en un suport sòlid.

La tècnica utilitzada, *Check-MDR CT103XL* (Check-Points, Wageningen, The Netherlands), mostra una bon rendiment [104] i els resultats s'obtenen en menys de 24 hores. Detecta gens de BLEEs, carbapenemases i Amp-c. Concretament de BLEEs detecta el grup CTX-M 1 amb els subgrups CTX-M-1, CTX-M-15, CTX-M-3, CTX-M-32, el grup CTX-M-2, el grup CTX-M-9 i el grup CTX-M8/25.

Cal tenir present, però, que esporàdicament apareixen noves BLEEs que no són detectades per aquest mètode.

4.4 Epidemiologia Molecular:

Les tècniques de tipificació molecular serveixen per a conèixer la relació genètica entres diferents aïllaments bacterians vinculats epidemiològicament i que deriven d'un microorganisme ancestral comú. A més a més també són tècniques que permeten diferenciar els aïllaments no relacionats que pertanyen a la mateixa espècie.

El grup d'aïllaments que descendeixen d'un ancestre comú o que procedeixen directament de la mateixa font o de la mateixa cadena de transmissió s'anomena clon o complex clonal.

Per tant són útils en la vigilància i control de brots perquè permeten conèixer la clonalitat entre aïllats, identificar reservoris i determinar vies de transmissió [105].

Existeixen diferents tipus de tècniques de tipificació molecular:

- Estudi de l'àcid desoxiribonucleic (ADN) cromosòmic mitjançant electroforesi en camp polsat (ECP)
- Amplificació d'àcids nucleics: anàlisi del nombre de còpies de determinades seqüencies d'inserció o de seqüencies repetides al llarg el cromosoma (rep-PCR) o a l'amplificació arbitrària de fragments genètics (AP-PCR).
- Seqüenciació d'ADN: estudis de restricció de l'ADN cromosòmic mitjançant a la seqüenciació de fragments interns de gens metabòlics molt conservats (*Multilocus sequence typing,* MLST).

4.4.1 Camp Polsat:

És una tècnica altament reproduïble i amb un poder discriminatori elevat, fet que el fa mètode de referència de tipificació per a la majoria de bacteris amb interès epidemiològic [105,106].

Es basa en la separació electroforètica en camp polsat d'ADN cromosòmic digerit mitjançant un enzim de restricció amb baixa freqüència de tall. La interpretació dels perfils d'ADN (pulsotips) es realitza aplicant els criteris de Tenover, et al [107] i és relativament senzill quan es treballa amb poques soques. Quan el nombre de soques és elevat o els perfils de bandes són complexes cal utilitzar un software específic.

Els inconvenients més destacats són el seu relatiu cost econòmic inicial, el procediment tècnic no automatitzat i el temps necessari per a obtenir i analitzar els resultats (una setmana mínim), el que fa que sigui una tècnica poc pràctica com a rutina del laboratori de microbiologia [105].

4.4.2 rep-PCR (repetitive-sequence-PCR)

Aquesta tècnica es fonamenta en la utilització de *primers* que hibriden de forma específica amb unes seqüències d'ADN repetitives, de funció desconeguda, i que es troben disperses per tot el genoma del bacteri [108].
Hi ha tres classes de seqüències repetitives i les més utilitzades en els estudis de brots són les seqüències REP (seqüències repetitives palindròmiques extragèniques).
La variabilitat en els perfils de bandes d'ADN generats mitjançant rep-PCR ve determinada pel nombre de seqüències repetitives i per la distància que hi ha entre elles.
És una tècnica senzilla, ràpida (menys de 2 dies), reproduïble i econòmica. Els perfils d'ADN presenten un nombre de bandes inferior al dels obtinguts per ECP, el que fa que l'anàlisi de la relació entre ells sigui relativament senzill [105].

Davant la sospita de l'inici d'un brot és important obtenir informació epidemiològica preliminar de forma ràpida i fiable i aquesta tècnica pot ser una eina adequada.

El sistema Diversilab® (bioMérieux, França) és un mètode flexible de rep-PCR que permet estudiar perfils de bandes de diferents microorganismes [109,110]. Presenta una alta reproductibilitat i permet una interpretació senzilla dels resultats gràcies a un *software* específic que analitza els perfils i agrupa en clones.

4.4.3 AP-PCR o RAPD:

És una tècnica d'amplificació a l'atzar d'ADN polimòrfic . És un mètode ràpid, poc laboriós i l'anàlisi de bandes és senzill, però té una baixa reproductibilitat que limita l'estudi en brots [105].

4.4.4 MLST:

Es basa en la seqüenciació parcial de 6 o 7 gens metabòlics molt conservats (*housekeeping genes*) que estan subjectes a escassa pressió selectiva [108].

A *K. pneumoniae* els gens seqüenciats estan descrits a la web de l'Institut Pasteur, París [111].

. *rpoB* (beta-subunitat de l' ARN polimerasa)

. *gapA* (gliceraldehid 3-fosfat deshidrogenasa)

. *mdh* (malat deshidrogenasa)

. *pgi* (fosfoglucosa isomerasa)

. *phoE* (fosforina E)

. *infB* (factor d'iniciació de la traducció 2)

. *tonB* (transductor de l'energia periplàsmica)

La majoria de les espècies bacterianes tenen una variació suficient en els gens metabòlics analitzats com per donar lloc a molts al·lels per cada un dels locus, permetent la diferenciació de mils de combinacions al·lèliques i utilitzant únicament 7 gens metabòlics. A cada seqüència obtinguda se li assigna un nombre d'al·lel diferent i cada soca es caracteritza per la combinació de les seqüències úniques dels al·lels en cada un dels seus loci, constituint el seu perfil al·lèlic o *seqüenciotip* (ST). L'assignació dels ST es fa utilitzant la base de dades de l'institut Pasteur abans citada.

És una tècnica molt laboriosa i costosa però els resultats obtinguts (seqüències tipus) són objectivables i es poden compartir informàticament amb altres laboratoris, el que permet comparar perfils o seqüències tipus entre aïllats de diferents països o continents [106].
El MLST té un poder discriminatiu baix pel que s'aplica principalment en estudis epidemiològics globals o a llarg termini per tal de conèixer l'estructura poblacional.

4.4.5 Seqüenciació massiva

Les tècniques de seqüenciació del genoma complet es van introduir al mercat a l'any 2005. La tècnica es basa en realitzar, de manera automatitzada, múltiples alineaments de les seqüències obtingudes amb les seqüències d'una base de dades interna que conté el software [112].

Referent a *K. pneumoniae*, es poden analitzar tant les seqüencies cromosòmiques com les plasmídiques [113].
En aquest moment, no és un mètode de tipificació rutinària en els laboratoris, però s'està introduint cada cop més gràcies a la seva gran utilitat [114,115]

A la Taula 5 es mostra un resum de les tècniques de tipificació molecular.

En aquest treball s'han utilitzat dos mètodes: la rep-PCR que és una tècnica ràpida de realitzar, i vàlida a l'estudi de possibles brots i amb un alt poder discriminatori i el mètode de MLST per a confirmar la diversitat o no de la nostra població.

Taula 5. Característiques tècniques de tipificació molecular.

Tècnica	Reproductibilitat	Poder de discriminació	Facilitat tècnica	Interpretació	Cost
Camp polsat	Excel·lent	Excel·lent	No automatitzada	Bona	Alt
rep-PCR	Bona	Excel·lent	Bona	Bona	Econòmic
AP-PCR o RAPD	Baixa	Excel·lent	Bona	Bona	Econòmic
MLST	Excel·lent	Baix	Laboriosa	Excel·lent	Alt
Seqüenciació massiva	Excel·lent	Excel·lent	Laboriosa	Excel·lent	Alt

. El temps de durada de les tècniques sempre dependrà del nombre de soques.

II. JUSTIFICACIÓ

Klebsiella pneumoniae productora de betalactamasa d'espectre estès (KpBLEE) s'ha associat tradicionalment a infeccions d'origen nosocomial i com a responsable de brots hospitalaris. Però recentment s'ha descrit un increment dels aïllaments de KpBLEE a la comunitat, especialment en relació a soques productores de l'enzim CTX-M-15.

Des de que ha emergit aquest enzim CTX-M-15 els brots nosocomials presenten unes característiques diferents dels causats per SHV o TEM: es distribueixen àmpliament per les diferents unitats d'un hospital general més que limitar-se a una de sola unitat, i la mortalitat es més baixa.

Darrerament, ha sorprès a la nostra epidemiologia local que alguns pacients amb infeccions per KpBLEE diagnosticades a l'hospital són definides com a comunitàries per l'absència de factors de risc associats amb l'atenció sanitària.

Per tant s'estudiarà aquest fenomen en el nostre entorn. L'estudi es du a terme a l'Hospital Universitari Mútua de Terrassa, hospital d'aguts de 400 llits amb una mitjana anual de 97.524 estades per una població de 350.000 habitants, i en els seus 9 centres d'assistència primària que atenen una població d'uns 300.000 habitants.

Els resultats d'aquests estudis podran ser útils per a entendre l'epidemiologia de la KpBLEE a la comunitat, les de debut hospitalari però de possible adquisició comunitària i les nosocomials. Això podria contribuir, tant, a la racionalització de les polítiques de control d'infecció com a les precaucions de contacte durant l'ingrés hospitalari i també poder dissenyar tractaments empírics en funció dels factors de risc de KpBLEE per tal d'optimitzar l'ús d'antibiòtics.

III. HIPÒTESI

Klebsiella pneumoniae productora de β-lactamasa d'espectre estès (KpBLEE) ha deixat de ser un patogen exclusivament nosocomial i responsable de brots hospitalaris a ser un patogen que també es troba a la comunitat, especialment a soques productores de CTX-M-15, en una proporció que desconeixem.

IV. OBJECTIUS

1. Determinar la prevalença d'infeccions urinàries per KpBLEE a la comunitat.

2. Determinar els factors de risc associats a les infeccions urinàries comunitàries.

3. Caracteritzar els tipus de β-lactamases de les KpBLEE causants d'infeccions urinàries.

4. Analitzar la dinàmica de transmissió de les KpBLEE en un hospital d'aguts.

5. Caracteritzar els tipus de β-lactamases de les KpBLEE hospitalàries.

V. METODOLOGIA

PRIMER ARTICLE:

1. Emerging extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* causing community-onset urinary tract infections: a case-control-study.

Àmbit:

Centres d'assistència primària de l'Hospital Universitari Mútua de Terrassa.

Període d'inclusió:

Gener 2010 – desembre 2014

Subjectes a estudi:

Pacients comunitaris adults amb clínica d'infecció del tracte urinari i aïllament de KpBLEE a l'urocultiu (una mostra per pacient)

Disseny:

Estudi retrospectiu cas control-control per a identificar els factors de risc associats a les infeccions del tracte urinari d'origen comunitari (ITU-Co) degudes a KpBLEE.

Per a evitar el risc de sobreestimar l'associació entre exposició a antibiòtics i resistència, s'han considerat dues poblacions per a la selecció de controls [116]:

- Controls amb ITU-Co per *Escherichia coli* no productora de β-lactamasa d'espectre estès (EcnoBLEE), l'etiologia més freqüent de les ITU. D'aquesta població per cada cas es van seleccionar dos pacients controls.
- Controls amb ITU-Co per *Klebsiella pneumoniae* no productora de β-lactamasa d'espectre estès (KpnoBLEE). També es van escollir dos pacients controls per cas .

Tots els pacients controls van seleccionar-se aleatòriament de les dues poblacions i es van aparellar amb els casos en la proporció 4:1 (2 EcnoBLEE i 2 KpnoBLEE per 1 cas) d'acord a edat, sexe i any de la ITU.

Variables a estudi:

- Demogràfiques
- Comorbiditats segons la taula de puntuació de Charlson [117]
- Estat funcional mitjançant l'índex de Barthel [118]
- Relació amb l'assistència sanitària [119]
- Antecedents d'infeccions del tracte urinari
- Antecedents de sondatge vesical
- Antecedents de tractaments antibiòtics previs.

L'estudi va ser aprovat pel Comitè local d'ètica de l'Hospital Universitari de Mútua de Terrassa.

Les recomanacions STROBE van ser seguides per a reportar els resultats de l'estudi.

Definicions:

- ITU: presència de símptomes relacionats amb el tracte urinari amb urocultiu positiu ($\geq 10^5$ ufc/mL).
- Bacteriúria asimptomàtica: pacients asimptomàtics amb urocultius positius (dones $\geq 10^5$ ufc/mL del mateix microorganisme en dues mostres, i en homes $\geq 10^5$ ufc/mL a una única mostra).
- ITU relacionada amb sondatge vesical: urocultiu positiu amb comptatge de $\geq 10^3$ ufc/mL.
- ITU recurrent: tres episodis en els últims 12 mesos o dos episodis en els últims 6 mesos. [120]
- Dependència funcional: definida per un índex de Barthel <60
- Antibiòtic previ: administració del menys una dosi d'antibiòtic durant els tres mesos previs. Específicament es va registrar l'exposició a quinolones i cefalosporines.
- Relació amb el medi sanitari (segons criteris de Friedman [119])
    - Infecció adquirida a l'hospital: >48 hores de l'admissió a l'hospital o antecedent d'ingrés hospitalari en els últims 14 dies.

- Infecció associada amb el sistema sanitari: presenta com a mínim un dels següents criteris:
  - Resident en un centre soci-sanitari, residència, en els 30 dies previs a l'episodi
  - Antecedent d'ingrés hospitalari de més de 8 hores en els 90 dies previs.
  - Pacients amb hemodiàlisi o amb tractaments intravenosos 30 dies abans de l'episodi
  - Cures de ferides, nutrició enteral o cures a domicili, 30 dies abans de l'episodi.
- Infecció comunitària: els altres casos

En aquest estudi tots els casos i controls van ser d'origen comunitari.

Mètodes microbiològics:

Les mostres d'orina es van sembrar en chromID CPS® agar (bioMérieux, Marcy-l'Étoile, France).

Les identificacions i antibiogrames mitjançant MicroScan® (Siemens, Munich, Germany).

Per a detectar les BLEEs es va realitzar la sinèrgia amb doble disc.

Els punts de talls de les CMIs es van basar en l' European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [103].

Es va realitzar l'extracció de l'ADN d'un cultiu pur de les soques de KpBLEE mitjançant QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) i la detecció dels gens de $bla_{CTX\text{-}M}$, bla_{SHV} i bla_{TEM} es va confirmar per un mètode de microarray (Check-MDR CT103 array; Check-Points, Wagenigen, The Netherlands).

Per a establir una possible relació clonal es va realitzar una rep-PCR Diversilab™ System (bioMérieux). Es van definir com a soques indistingibles les que compartien un ≥97% de similitud i les íntimament relacionades un ≥95%.

Anàlisi estadística:

Tota l'anàlisi estadística es va fer utilitzant STATA Statistical Software Release 14 (StataCorp LP, College Station, TX).

La prevalença anual es va calcular usant com a denominador el nombre total d'aïllaments de *K. pneumoniae* aïllats a urocultius de pacients ambulatoris i com a numerador el total de KpBLEE aïllades a mostres d'orina (una soca per pacient per any).

La tendència anual es va analitzar mitjançant un model d'ajustament lineal.

Les variables categòriques es presenten com a nombres o percentatges i les variables continues com a mitja i desviació estàndard o la mediana i rang interquartil.

La regressió logística condicional es va utilitzar per a computar l'odds ratio crua amb un interval de confiança del 95% i també per a l'anàlisi multivariat.

Començant amb totes les variables que van mostrar una tendència cap a l'associació ($p < 0.2$), es va identificar el model multivariat més adequat mitjançant el procediment de regressió logística de subconjunts. Es va seleccionar el model d'informació d'Akaike més baix [121].

Les diferències es van considerar estadísticament significatives at two-side *p*-value de <0.05 level.

El test χ^2 es va utilitzar per a detectar diferències entre el patró de sensibilitat antimicrobiana entre els casos de KpBLEE i els controls per KpnoBLEE.

SEGON ARTICLE:

2. High clonal diversity of ESBL-producing *Klebsiella pneumoniae* isolates from clinical samples in a non-outbreak situation. A cohort study.

Àmbit:

Hospital Universitari Mútua de Terrassa

Període d'inclusió:

2015

Disseny:

Estudi prospectiu de cohorts

Subjectes a estudi:

Tots els pacients adults nous consecutius amb aïllament de KpBLEE a qualsevol mostra clínica obtinguda per pràctica de rutina clínica (una mostra/un pacient).

Els pacients amb antecedents d'infecció / colonització prèvia per KpBLEE i els pacients ingressats a la Unitat de Cures Intensives van ser exclosos .

Definicions:

- "Pacient índex": pacient ingressat amb un primer aïllament de KpBLEE a una mostra clínica-
- "Pacient contacte": persona que comparteix la mateixa habitació durant ≥24 hores amb un pacient índex sense precaucions de contacte.
- "Transmissió creuada": quan un pacient que comparteix la mateixa habitació o planta durant ≥48 hores amb un cas índex és portador d'una KpBLEE clonalment relacionada amb aquest, durant un temps màxim de 4 setmanes.
- Relació amb el medi sanitari (segons criteris de Friedman [119]):
    - Infecció adquirida a l'hospital: >48 hores de l'admissió a l'hospital o antecedent d'ingrés hospitalari en els últims 14 dies.

- Infecció associada amb el sistema sanitari: presenta com a mínim un dels següents criteris:
    - Resident en un centre soci-sanitari, residència, en els 30 dies previs a l'episodi.
    - Antecedent d'ingrés hospitalari de més de 8 hores en els 90 dies previs.
    - Pacients amb hemodiàlisi o amb tractaments intravenosos 30 dies abans de l'episodi.
    - Cures de ferides, nutrició enteral o cures a domicili, 30 dies abans de l'episodi.
- Infecció comunitària: els altres casos.

Control d'infecció:

Tots els pacients diagnosticats es van posar en precaucions de contacte, que incloïen estar en una habitació sol o acompanyat per un altre pacient portador de KpBLEE i ús de guants i bata per part del personal sanitari.

Els pacients índexs van ser estudiats al moment de la primera detecció per a conèixer l'estat de portador, mitjançant un frotis rectal i mostra d'orina si eren portadors de sonda vesical.

No es van seguir polítiques actives de descolonització.

Els estàndards bàsics de control d'infecció inclouen una adequada higiene de mans, tal com indiquen les guies de l' Organització Mundial de la Salut (OMS) [122]. Durant el 2015, amb un projecte de promoció de la higiene de mans a tot l'hospital, el compliment va ser del 64%. Durant el període de l'estudi es va dur a terme un rigorós procés de neteja ambiental que incloïa la neteja de les habitacions amb pacients amb KpBLEE dos cops al dia amb detergents i una neteja terminal a l'alta.

L'equip de control d'infecció feia un seguiment de tots els pacients ingressats amb aïllament de KpBLEE recollint prospectivament:

- Dades demogràfiques.

- Tipus de mostra.
- Relació amb l'assistència sanitària.
- Moviments dins de l'hospital (plantes, habitacions).

Mostres ambientals:

Durant l'any de l'estudi es van realitzar estudis de colonització ambiental a algunes de les habitacions ocupades per pacients infectats o colonitzats per KpBLEE. De cada habitació s'obtenien 4 mostres: aixeta de la pica, superfície de la pica, cunya i renta-cunyes [123].

Les mostres per a cultiu es van obtenir fregant repetidament una gasa humitejada amb caldo de tioglicolat a la superfície a estudiar, després es posaven en un contenidor estèril afegint 10mL de caldo de tioglicolat. Els contenidors s'incubaven 24 hores a 37ºC i després es sembraven en una placa de ChromID ESBL (bioMérieux) [31,124].

Mètodes microbiològics:

Les identificacions i els estudis de sensibilitat es van fer mitjançant el sistema Vitek2 (bioMérieux).

Es van utilitzar els punts de tall de l'EUCAST per a la interpretació dels resultats.

Es va confirmar la producció de BLEE mitjançant el mètode de la doble difusió en disc.

La caracterització de les BLEEs es va fer per una PCR comercial (Check-MDR CT103XL, Hain).

La relació genètica de les soques de KpBLEE es va determinar mitjançant una rep-PCR (Diversilab System, bioMérieux), seguint les recomanacions del fabricant. Es van analitzar mitjançant el software de Diversilab utilitzant el coeficient de correlació de Pearson. Es va definir que dues soques eren idèntiques quan presentaven >95% de similitud.

La tècnica de Multilocus sequence typing (MLST) es va dur a terme utilitzant 7 gens metabòlis molt conservats (*housekeeping genes)*: *gapA, infB, mdh, pgi, phoE, rpoB* i *tonB* [106].

El protocol del procediment del MLST es troba a les bases de dades de l'Institut Pasteur [111].

Les relacions filogenètiques entre els diferents ST trobats a l'estudi van ser establertes per Phyloviz utilitzant l'algoritme goeBURST [125].

VI. RESULTATS

PRIMER ARTICLE:

1. Emerging extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* causing community-onset urinary tract infections: a case-control-study.

1. Estudi cohort retrospectiu:

La prevalença de les infeccions urinàries d'origen comunitari per *Klebsiella pneumoniae* productora de β-lactamasa d'espectre estès va augmentar d'un 2,4% al 2010 al 10,3% el 2014 (p=0.01), mentre que les causades per *K. pneumoniae* no BLEE (KpnoBLEE) van restar estables (7,5% el 2010 i 8,5% el 2014).

2. Estudi cas-control-control:

Durant el període d'estudi es van aparellar un total de 83 casos d' infeccions del tracte urinari d'origen comunitari (ITU-Co) per KpBLEE amb 319 controls d' ITU-Co: 164 degudes a *E. coli* no BLEE (EcnoBLEE) i 155 a KpnoBLEE.

L'anàlisi univariat va mostrar que els factors de risc per ambdues poblacions eren: ser resident d'un centre soci-sanitari o residència, admissió prèvia a un hospital, presentar comorbilitat (índex de Charlson), infeccions del tracte urinari de repetició, portador de sonda vesical permanent i exposició prèvia a tractament antibiòtic (cefalosporines, fluorquinolones o altres antibiòtics).

L'anàlisi multivariat es va fer separadament per cada un dels dos grups control. Les variables independents associades a ITU-Co per KpBLEE van ser: ser resident d'un centre soci-sanitari o residència [odds ratio (OR) = 8.8, 95% interval de confiança (IC) 2.6-29.4] i ús previ de cefalosporines (OR = 4.01, 95% IC 1.8-9.2).

La síndrome clínica va ser desconeguda en quasi el 50% dels casos i en 2/3 dels grups control i quan es va identificar la més freqüent en tots els grups va ser la cistitis, seguida de bacteriúria asimptomàtica, infecció relacionada amb sonda vesical, pielonefritis i prostatitis en tots els grups.

El patró de resistència va demostrar que les KpBLEE eren molt més resistents als antibiòtics estudiats i que el 86.7% eren multiresistents.

Quasi 2 /3 dels casos (63,8%) van ser d'origen estrictament comunitari.

3. Caracterització molecular:

Dels 83 aïllaments de KpBLEE, se'n van caracteritzar 29 (34,9%) mitjançant rep-PCR i es van trobar 17 patrons clonals diferents. D'aquestes 29 soques, 23 (79,3%) produïen CTX-M-15, 4 (13,8%) CTX-M-9 i 2 (6,9%) SHV-238S.

SEGON ARTICLE:

2. High clonal diversity of ESBL-producing *Klebsiella pneumoniae* isolates from clinical samples in a non-outbreak situation. A cohort study.

Durant 2015, es van identificar seixanta casos consecutius de pacients amb aïllament de KpBLEE a l'Hospital Universitari Mútua de Terrassa.

Per ordre de freqüència, la procedència de les mostres va ser orina (47, 78%), ferides quirúrgiques (6, 10%), sang (6, 10%) i una mostra respiratòria (2%).

Les mostres es van obtenir a 32 pacients d'urgències, a 16 pacients ingressats a serveis quirúrgics i a 12 de serveis mèdics.

Els nous casos es van detectar amb una freqüència mensual de 2,5 (rang 0-6) i no es va detectar cap cas amb relació temporo-espacial que segons la definició establerta complís els criteris de transmissió creuada.

Les infeccions (78,3%) i les colonitzacions (21,7%) es van classificar com a adquirides a l'hospital (52%), relacionades amb l'assistència (40%) i adquirides a la comunitat (8%).

De les mostres relacionades amb l'assistència sanitària, 11 (17%) eren residents de centres soci-sanitaris o residències. En concret, a dues residències, es van identificar dos casos, però no es va trobar cap relació temporal ni genètica entre els aïllaments de cada centre.

Es va detectar una elevada diversitat genètica. Mitjançant rep-PCR (Diversilab®) les 60 soques es van classificar en 36 patrons i sols 4 d'aquests incloïen tres o més aïllaments. Per MLST es van identificar 16 *seqüenciotips* (ST). Els més prevalents van ser ST170 (23%), ST405 (21%) i ST392 (16%). Tots aquest junts representen el 60% dels aïllaments.

Entre els 31 casos d'adquisició hospitalària o nosocomials es van identificar 19 patrons diferents de rep-PCR i 8 STs diferents. Els més freqüents van ser ST170 (26%),

ST405 (26%) i ST392 (23%). No hi havia relació temporo-espacial entre les soques aïllades.

Dels casos relacionats amb l'assistència sanitària, 22 de 24 tenien patrons de rep-PCR diferents i 11 STs. En aquests la freqüència va ser: ST170 (25%), ST405 (21%) i ST392 (13%).

Cinc casos comunitaris es van incloure i els 5 tenien diferents patrons de rep-PCR i de MLST. Dos ST comunitaris (ST70 i ST307) també es van identificar a dues soques relacionades amb l'assistència i a tres soques nosocomials.

El mecanisme genètic detectat a totes les KpBLEE estava associat amb la presència de gens *bla* $_{\text{CTX-M}}$. La majoria de CTX-M pertanyien al grup 1: CTX-M-15 (58 soques) i CTX-M-32 (una soca). Una altra soca pertanyia al grup 9. No es va detectar cap tipus de carbapenemasa.

El patró de resistència antibiòtica va mostrar que totes les soques eren resistents a cefotaxima i ceftazidima i la caracterització fenotípica va mostrar diferències de resistència entre aïllaments amb el mateix *seqüenciotip*.

A l'estudi realitzat de mostres ambientals a 4/32 (12,5%) es va detectar KpBLEE a 3 habitacions. Una habitació tenia 2 superfícies positives (pica del pacient i aixeta del *renta-cunyes*) amb idèntic patró de rep-PCR però aquest patró no es va identificar a cap pacient. Les altres 2 mostres positives van ser aïllades a diferents habitacions. La primera era una mostra de la superfície del voltant de la pica i tenia el mateix patró que una soca aïllada en un pacient que prèviament havia ocupat la mateixa habitació; la segona mostra presentava un patró diferent del pacient que havia ocupat l'habitació.

ARTICLES

PRIMER ARTICLE:

Emerging extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* causing community-onset urinary tract infections: a case-control study.

Lucía Boix-Palop, Mariona Xercavins, Cristina Badía, Meritxell Obradors, Montserrat Riera, Núria Freixas, Josefa Pérez, Mónica Rodriguez-Carballeira, Javier Garau, Esther Calbo.

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Contribució de doctoranda:

La doctoranda ha participat en el disseny de l'estudi, ha dut a terme junt amb altres autores els estudis microbiològics, la recollida de dades, l'anàlisi dels resultats i la redacció de l'article.

Emerging ESBL-producing *Klebsiella pneumoniae* causing community-onset urinary tract infections. A case-control-control study.

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Abstract

The aim of this study was to determine the epidemiology and risk factors associated with community-onset urinary tract infections (CO-UTIs) due to extended-spectrum ß-lactamase (ESBL)-producing *Klebsiella pneumoniae* (ESBL-Kp).

A cohort study, including all consecutive patients with *K. pneumoniae* CO-UTI identified from January-2010 to December-2014, was conducted. Patients with CO-UTIs due to ESBL-Kp were then included as cases in a retrospective case-control-control study; controls were outpatients with CO-UTI caused by non-ESBL-producing *E. coli* and *K. pneumoniae* (non-ESBL-Ec, non-ESBL-Kp, respectively). Each control was matched in a 2:1 ratio according to age, sex and year of isolation. Genotyping confirming ESBL was performed with multiplex-PCR and sequencing.

The prevalence of ESBL-Kp CO-UTIs, calculated within all *K.pneumoniae* CO-UTIs, increased from 2.4% in 2010 to 10.3% in 2014 (p=0.01). Among cases, 63,8% were truly community-acquired and CTX-M-15 was the predominant β-lactamase enzyme-type (79.3%). A total of 83 cases and 319 controls were studied. Being a nursing home resident [odds ratio (OR) = 8.8, 95% confidence interval (CI) 2.6–29.4] and previous cephalosporin use (OR =4.01, 95% CI 1.8–9.2) were risk factors independently associated with CO-UTI due to ESBL-Kp.

In conclusion, the prevalence of CO-UTIs due to ESBL-Kp is increasing. In most cases, ESBL-Kp CO-UTIs are community-acquired and produce CTX-M-15 β-lactamase. Exposure to cephalosporins and to being a nursing home resident were risk factors associated with ESBL-Kp CO-UTIs. CTX-M-15 producing *K. pneumoniae* isolates are emerging in the community.

Key words: *Klebsiella pneumoniae*; extended-spectrum ß-lactamase; ß-lactamase CTX-M-15; community-onset; urinary tract infection.

1. INTRODUCTION

Antimicrobial resistant *Klebsiella pneumoniae* has been traditionally recognized as an important nosocomial pathogen affecting mainly severely ill patients. Since the first identification of an extended spectrum ß-lactamases (ESBL) able to hydrolyze oxymino cephalosporins in the early 1980s, third generation cephalosporin resistance has been mainly due to the ESBL production (1).

ESBLs are plasmid-mediated enzymes that have the ability to hydrolyze narrow and expanded-spectrum cephalosporins and aztreonam, but do not affect cephamycins and carbapenems. These enzymes are inhibited by the so-called 'classical' ß-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. Frequently, ESBL plasmids also encode other resistance determinants involving fluoroquinolones, cotrimoxazole and aminoglycosides (2).

The first types of ESBLs described were derivatives of the TEM-1, TEM-2 and SHV-1 enzymes, mainly in *K. pneumoniae* strains associated with nosocomial outbreaks (1). With the emergence of the CTX-M enzymes in the late 1980s, the epidemiology changed and most ESBL-producing strains were found in *E. coli* community acquired infections (1, 3).

In recent years, a new trend is being observed among *K. pneumoniae*: most nosocomial isolates are now CTX-M type, specifically CTX-M-15. The epidemiology has somehow changed after the emergence of CTX-M-15 enzyme type among *K. pneumoniae*. Reported nosocomial outbreaks caused by CTX-M-15 producing *K. pneumoniae* are frequently described as widely distributed in general hospital wards rather than limited to specific units, and mortality seems to be lower than that previously described for SHV and TEM outbreaks (4). Furthermore, CTX-M-15-producing *K. pneumoniae* has been also increasingly recognized in the community (5). Factors driving this increase are not clear. Although several studies to identify risk factors for community acquired ESBLs-producing *Enterobacteriaceae* have been published (6, 7), most have focused exclusively on CTX-M producing *E. coli*, and little is known about risk factors associated with CTX-

M-15 producing *K. pneumoniae* infections (8).

The aim of the present study was to determine the prevalence, clinical features and risk factors associated with community-onset urinary tract infections (CO-UTIs) due to ESBL-producing *K. pneumoniae* (ESBL-Kp), and to study the molecular epidemiology of ESBL carrying isolates in the CTX-M era.

2. MATERIALS AND METHODS:

2.1 Setting, patients and study design

The study was carried out in the Hospital Universitari Mútua de Terrassa, a 500-bed teaching hospital (mean annual admission of 24,000 patients, serving 8 primary care centers).

Adult outpatients were identified retrospectively through the Microbiology Laboratory record, which receives samples from the hospital, a nearby chronic-care hospital, and the primary care centers of an area of *circa* 950,000 inhabitants. Only samples obtained from non-hospitalized patients in the primary care centers were included.

All urinary isolates of *K. pneumoniae* with reduced susceptibility to Cephalosporins identified in the Community Department of the Microbiology Laboratory from January 2010 to December 2014 were included. Genotyping confirming ESBL isolates was performed with multiplex PCR. A retrospective case-control-control study was performed to identify risk factors associated with community-onset urinary tract infections (CO-UTIs) due to ESBL-producing *K. Pneumoniae* (ESBL-Kp). Cases were defined as outpatients with CO-UTI due to ESBL-Kp (one sample per patient) during the study period. In order to avoid the risk of overestimating the association between antibiotic exposure and resistance, two populations were considered for controls selection (9): (1) controls with a CO-UTI syndrome uninfected by *K. pneumoniae* and (2) controls infected with a susceptible strain of *K. pneumoniae*. The first control group was made up of

patients with CO-UTI due to non ESBL-producing *E.coli*, the commonest etiology of CO-UTI, considered as general population (hereafter referred to as non-ESBL-Ec controls). From this population, 2 control patients per case patient were chosen. The second base population was constituted of patients with CO-UTI due to susceptible *K. pneumoniae* (hereafter referred to as non-ESBL-Kp controls); for this population, 2 control patients per case patient were also chosen. Control patients were randomly selected from both populations and matched to case patients in a ratio 4:1 (2 non-ESBL-Ec and 2 non-ESBL-Kp controls:1 case) according to age, sex and year of isolation.

Data collected concerning patients included demographics, comorbidities based on Charlson score (10), functional status measured by Barthel Index (11), health care relation, recurrent UTI, permanent urinary catheter, clinical characteristics and previous antibiotic treatment. Data were collected from the medical charts and electronic medical records of hospital and primary care centers.

The study was approved by the local ethics committee. Due to the observational character of the study, written informed consent was not required.

STROBE recommendations were followed to strengthen the reporting of results of this study (supplementary material, Table S1).

2.2 Definitions

UTI was defined by the presence of symptoms related to the urinary tract and a positive urine culture ($\geq 10^5$ CFU/mL). Asymptomatic patients with positive urine cultures were considered to have asymptomatic bacteriuria (women $\geq 10^5$ CFU/mL of the same uropathogen in two samples, men $\geq 10^5$ CFU/mL in only one sample, $\geq 10^2$ CFU/mL if obtained from permanent urinary catheter). Recurrent UTI was defined as three episodes of UTI in the last 12 months or two episodes of UTI in the last six months, including the current episode (12). Functional dependence

was defined as Barthel Index < 60. Previous antibiotic use was defined as administration of at least a single dose of antibiotic during the previous three months; previous exposure to quinolones and cephalosporins was specifically identified and recorded.

Health care relation was defined according to *Friedman et al* (13). Hospital-acquired infection was defined as an infection acquired during hospital care that was not present or incubating at admission (infections occurring >48 h after admission were considered nosocomial) or in a patient discharged from hospital in the previous 14 days. Healthcare-associated infection was diagnosed if the patient fulfilled at least one of the following criteria: (i) resided in a nursing home or long-term care facility in the 30 days before the episode; (ii) hospitalized in an acute care hospital for ≥48 h, 90 days before the episode; (iii) attended a hospital or hemodialysis clinic or received intravenous therapy, 30 days before the episode; and/or (iv) received intravenous therapy, wound care, enteral nutrition or healthcare at home, 30 days before the episode. Otherwise the infection was considered as community acquired. In the present study, all included cases and controls were non hospital-acquired infections and therefore they were considered as community-onset episodes.

ESBL-producing *K. pneumoniae* strains were considered multiresistant if they were non-susceptible to at least one agent in three or more antimicrobial categories as defined by *Magiorakos et al* (14).

2.3 Microbiological studies

Samples were plated onto ChromID CPS agar (BioMérieux). Identification and antimicrobial susceptibility testing were performed using MicroScan (Siemens). Double-disk synergy test was used to detect ESBLs. MIC breakpoints for resistance were based on EUCAST criteria (15).

Genotyping confirmed ESBL isolates was performed with the multiplex PCR application using a

commercially available kit (MARCA). Amplification products are hybridized using a microarray and visualized by colorimetric detection (Check-MDR CT103 array, Wageningen, The Netherlands,) for detection of bla_{CTX-M}, bla_{SHV}, bla_{TEM} genes, according to the manufacturer's instructions. A DNA extraction from a pure culture of each isolate was made using the mini-kit QIAamp DNA (Qiagen, Sample & Assay Technologies)

Genotypic procedure Rep-PCR experiments were performed using the DiversiLab system (bioMérieux, Marcy-l'Etoile, France) according to the manufacturer's instructions for the molecular epidemiological studies. Results were analyzed with DiversiLab software using the Pearson correlation coefficient method to determine distance matrices and with the unweighted pair group method with arithmetic, pattern matching to determine the clonal relationships and to create dendrograms. A cluster of closely related isolates was defined as isolates sharing ≥95% similarity and indistinguishable isolates of ≥97%.

2.4 Statistical analysis

All statistical analyses were performed using the STATA RELEASE 14 software (StataCorp LP, College Station, TX, USA). The annual prevalence was calculated using as denominator the total number of *K. pneumoniae* isolated from urinary samples from non hospitalized patients and numerator the total number of ESBL producing *K. pneumoniae* isolated in urinary samples (one isolate from each non hospitalized adult patient included) per year. The annual trend was analyzed using a linear adjustment model. Categorical variables are presented using counts and percentages and continuous variables as means and standard deviation (SD) or medians and interquartile range (IQR). Conditional logistic regression was used to compute crude odds ratio (OR) and 95% confidence intervals (CI). Multivariate analysis was also performed by conditional logistic regression. Starting with all variables that showed a trend towards an association ($p <$

0.2), a best subset regression procedure was used to identify the most suitable and parsimonious multivariate model, i.e. the one with the lowest Akaike information criterion, which is a well-known parameter of the goodness of fit of the model (16). Differences were considered statistically significant at the two-sided $p < 0.05$ level. To detect differences between the antibiotic susceptibility pattern of cases and *K. pneumoniae* controls the chi-square test was used.

3 RESULTS

3.1 **Retrospective cohort study**

The prevalence of community-onset UTIs due to ESBL- producing *K. pneumoniae* in our area, calculated within all *K.pneumoniae* CO-UTIs, increased from 2.6% in 2010 (6 out of 253 CO-UTIs caused by ESBL-*K.pneumoniae*) to 10.3% in 2014 (30 out of 291) (p=0.01), while the prevalence of CO-UTIs due to non-ESBL *K. pneumonia*, calculated within all CO-UTIs, remained stable (from 7.5% in 2010 to 8.5% in 2014, p=0.08) (figure 1).

3.2 Case-control-control study

During the study period, a total of 83 cases of CO-UTI due to ESBL-producing *K. pneumoniae* and 319 matched controls of CO-UTI (164 due to non-ESBL-Ec and 155 due to non-ESBL-Kp*)* were analyzed; 13 (3.9 %) controls had to be excluded (4 because of missing data on clinical charts and 9 due to previous episodes of infections caused by ESBL-producing *Enterobacteriaceae*).

Table 1 shows the results of the univariate analysis. The interactions tested for both populations were: functional dependence, to be a nursing home resident, previous hospital admission,

Charlson score, recurrent UTI, permanent urinary catheter and previous exposure to antibiotic treatment: cephalosporins, fluoroquinolones or other antibiotics.

Multivariate analysis was done separately for each of both control groups. With regard to the non-ESBL-producing *K. pneumoniae* population, the following variables were introduced into the multivariate analysis: functional dependence, to be a nursing home resident, previous hospital admission, recurrent UTI, previous exposure to cephalosporins and to fluoroquinolones. The variables independently associated with CO-UTI due to ESBL- producing *K. pneumoniae* are shown in Table 2. With regard to the non-ESBL-producing *E.coli* population, the following variables were introduced into the multivariate analysis: functional dependence, to be a nursing home resident, previous hospital admission, Charlson score, recurrent UTI, permanent urinary catheter and exposure to cephalosporins and to fluoroquinolones. The variables independently associated with CO-UTI due to ESBL- producing *K. pneumoniae* are also shown in Table 2.

The clinical syndrome was unknown in nearly 50% of cases and in 2/3 of both control groups. The most prevalent syndrome was cystitis, followed by asymptomatic bacteriuria, catheter-related UTI, pyelonephritis and prostatitis in all groups.

The antibiotic susceptibility pattern for cases and *K. pneumoniae* controls is shown in Table 3. ESBL-producing *K. pneumoniae* exhibited much higher resistance to all antimicrobials tested. Eighty-seven per cent (72/83) of the ESBL-producing *K. pneumoniae* were multidrug-resistant.

3.3 Molecular characterization

Twenty-nine out of 83 (34.9%) isolates were available for molecular characterization. Seventeen different clonal patterns, designated as A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P and Q, were identified. The most prevalent clonal pattern was F with 6 isolates, followed by E and L with 3

and 2 isolates, respectively. Twenty-three isolates (79.3%) produced CTX-M-15, 4 (13.8%) CTX-M-9 and 2 (6.9%) SHV-238S.

4 DISCUSSION

ESBL-producing *K. pneumoniae* has been traditionally recognized as a common cause of nosocomial infections and hospital outbreaks. Recent data suggest that the presence of ESBL-producing *K. pneumoniae*, and specifically CTX-M15 producing strains, might be emerging in the community (4). However, scarce information is available on the epidemiology of these community-acquired infections and the specific risk factors for CTX-M producing *K. pneumoniae* have not been well identified.

The present study provides some insights into this evolving epidemiology. Here we report a relevant increase in the prevalence of ESBL-producing *K. pneumoniae* causing CO-UTI in a large health area, in parallel with an increase in the prevalence of the CTX-M-15 enzyme type among *K. pneumoniae* isolates. These findings are consistent with recent reports, confirming the increased presence of ESBL-producing *K. pneumoniae* in the community (17, 18, 19).

It is noteworthy that almost two thirds of the case patients in our study had not been hospitalized during the previous three months, had not been in contact with hospital outpatient services, nor had they been in nursing homes, so their infections can be considered truly community acquired. Moreover, more than three quarters of the ESBL-producing *K. pneumoniae* strains were multiresistant. Specifically, most were resistant to fluoroquinolones and trimethoprim/sulfamethoxazole.

The high prevalence of CO-UTI due to CTX-M-15 producing *E. coli* is an established fact. However, for *K. pneumoniae*, until now, CTX-M-15 production in the community was anecdotic. The results showed in the present study represent a new and worrisome scenario in the

community setting: the second most frequently isolated agent causing CO-UTI is now also harboring CTX-M15, an enzyme until recently associated with *E. coli*. On the other hand, some patients with infections due to *K. pneumoniae* harboring ESBL seen in the hospital setting are now epidemiologically considered as community-acquired, having occurred among patients with no discernible healthcare-associated risk factors. If these findings are confirmed in other areas, this may change our traditional epidemiological view. In fact, hospital outbreaks originating from a community source of *K. pneumoniae* harboring ESBL have been already reported (20, 21).

To investigate risk factors for resistance among patients with infections due to a specific microorganism, the control group should be chosen among patients with the same susceptible bacteria of the same species. However, such a design may overestimate the importance of previous antimicrobial use because patients who had received antimicrobials would probably be underrepresented in the control population. This can be avoided by choosing the control patients from among all patients at risk, although some of the identified risk factors might then be nonspecifically associated with the risk of developing an infection caused by the susceptible organism (9). For this reason, a case-control-control design was proposed. A similar design has been used to identify risk factors for bloodstream infections caused by ESBL-producing *E. coli* (22).

To be a nursing home resident and previous use of cephalosporins were risk factors in both populations, indicating that they are truly associated with CO-UTI due to ESBL-producing *K. pneumoniae*. Previous use of quinolones was an specific risk factor to CO-UTI due to ESBL-producing *K. pneumoniae* in the non-ESBL-Kp control group, but taking into account that it was not found in the non-ESBL-Ec control group , the association may oversestimate the importance of the previous use of quinolones.

These two risk factors identified in our study, are similar to those previously reported for community-acquired infections caused by other *Enterobacteriaceae*, mainly ESBL-producing *E. coli* (3, 6, 23, 24). However, until now, ESBL-producing *E. coli* and *K. pneumoniae* have been considered to present differences in epidemiological risk profiles, representing differences in transmission dynamics (8).

These findings may suggest that the acquisition of CTX M 15 enzyme type by *K. pneumoniae* in community isolates may resemble the epidemiological phenomenon described years ago with *E .coli*, suggesting that the interface between hospitals and community is becoming blurred. There are many examples of the presence of *K. pneumoniae* in the community: there are reports of faecal carriage of ESBL-producing *K. pneumoniae* in outpatients and healthy individuals in many geographical areas (5) (25), and tap water and treated waste water, as well as food have been identified as the possible sources of *K. pneumoniae*-harboring CTX-M-15 (26, 27).

CTX-M-15 enzyme type was the most common ESBL type identified among cases in the present study. CTX-M enzymes have epidemically expanded among *Enterobacteriaceae* and are now the dominant ESBL type among clinical isolates, mainly among *E. coli* (28). An important factor in their global dominance is the wide dissemination of bacterial clones producing CTX-M-type ESBLs. In fact, the clonal dissemination of CTX-M-15-producing *E. coli* belonging to phylogenetic group B2 and sequence type (ST) 131 has been identified as the most prevalent in many countries (29). Remarkably, no clonal dissemination was detected in our cohort contrary to the community-acquired ESBL *E. coli* epidemics described previously. Probably, the rapid emergence of CTX-M-15 as one of the most important epidemic ESBLs in *E. coli* has generated a large genetic reservoir from which other species, such as *K. pneumoniae*, could easily acquire this resistance gene (30). However, with *K. pneumoniae* it seems that resistance is not restricted

to a few genetic backgrounds and that is a phenomenon of multiple emergence rather than the spread of a few clones (31)

Our study has some limitations. Data were collected retrospectively from medical charts and electronic medical records in hospital and primary care settings, so it was difficult to delineate with accuracy the clinical syndrome due to missing information. We did not include patients from Emergency Department, which may have contributed to an underrepresentation of community-acquired cases. Matching was performed according to age and gender, in order to avoid confounding with the comorbidities and the highest prevalence of low UTI in women, respectively. In consequence, these two variables have not been investigated as risk factors. Health care relation was defined according to the criteria of *Friedman et al*. Although the criteria were not modified in accordance with the epidemiology of the UTI, relevant variables (recurrent UTI, permanent urinary catheter) were collected. Despite our best efforts, only a third of samples were available for enzyme characterization, and plasmid analysis was not performed. Finally, our study was conducted in an urban teaching institution with a very large outpatient population, and the results cannot be extrapolated to other settings.

Despite the above limitations, our study has shown a remarkable increase in the prevalence of ESBL-producing *K. pneumoniae* in the community and that previous exposure to cephalosporins, as well as being a nursing home resident are clearly linked to the isolation of ESBL-producing *K. pneumoniae* causing UTI in the outpatient population. CTX-M-15 enzymes appear to have replaced older types of ESBLs and to have become the predominant enzymes among several different *K. pneumoniae* clones.

The results of our study may have some practical consequences. First, interventions directed at reducing the use of cephalosporins for the treatment of CO-UTIs whenever possible should be pursued. Second, when treatment protocols are designed, it might be prudent to consider empirical therapy with agents active against ESBL-producing organisms in high risk-patients with

severe community-acquired urinary tract sepsis. Third, hospital infection control measures should contemplate the evolving epidemiology of CTX-M15 *K. pneumoniae*.

5 CONCLUSIONS

The prevalence of community onset-UTI due to ESBL-Kp is increasing. CTX-M-15 enzymes appear to have replaced older types of ESBLs in *K. pneumoniae* and to have become the predominant enzymes among several different clones. Remarkably, almost two thirds of cases were truly community-acquired. The identified risk factors for CO-UTI due to ESBL-Kp were exposure to cephalosporins and to be a nursing home resident.

6 CONFLICTS OF INTEREST:

J.G. has accepted grants from Vifor Pharma, Bayer and Pfizer, and speaking engagements and conference invitations from Astellas, AstraZeneca, Novartis, Pfizer, GSK, Bayer, Vifor Pharma, Cubist, Durata and Theravance. E. C. has accepted grants, speaking engagements and conference invitations from Astellas, AstraZeneca, Novartis, Pfizer and MSD. L.B. has accepted conference invitations from Astellas, Pfizer and MSD. All other authors: none to declare.

7 FUNDING:

No specific funding has been received. This study was conducted as part of our routine work.

8 ETHICAL APPROVAL:

The study was approved by the local ethics committee.

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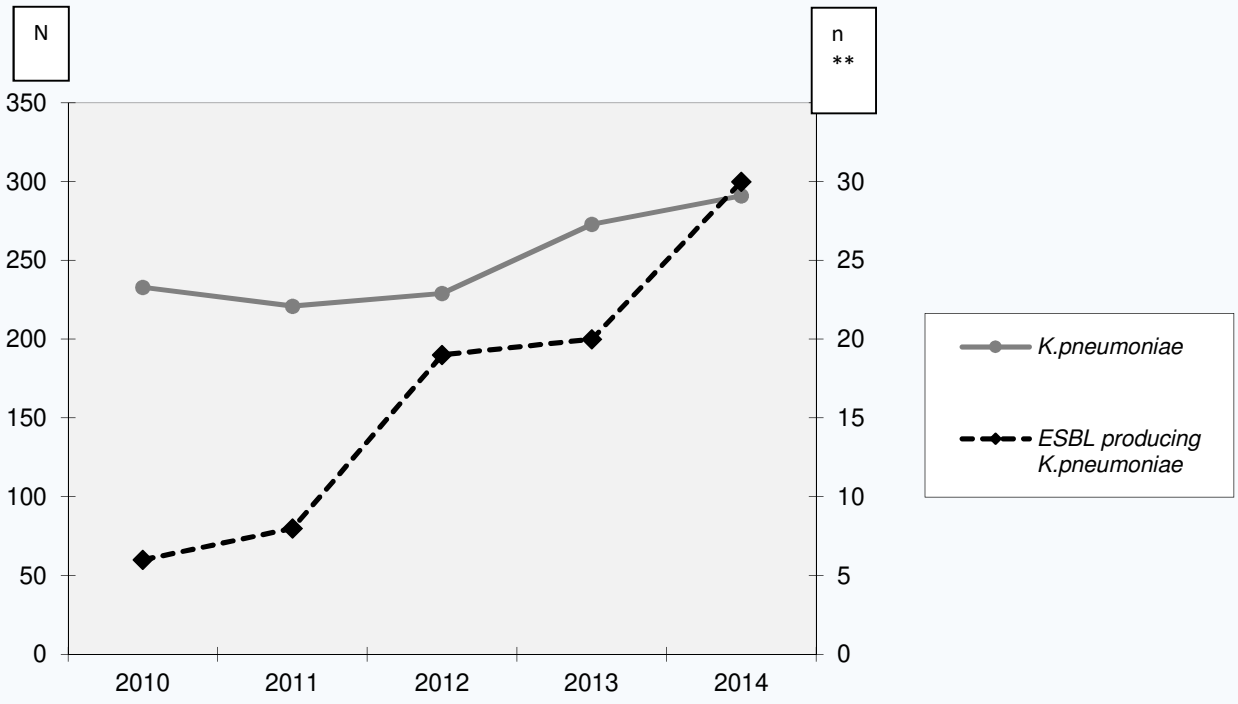
Figure 1. Annual cases of CO-UTI due to ESBL producing *K. pneumoniae* (dashed lines) and non ESBL producing *K. pneumoniae* (solid lines).



*N: Absolute annual number of CO-UTI due to non ESBL-producing *K. pneumoniae*

**n: Absolute annual number of CO-UTI due to ESBL-producing *K. pneumoniae*

Table 1. Univariate Analysis of Risk Factors for Case Patients with CO-UTI due to ESBL-producing *K. pneumonia,* Compared with Patients with CO-UTI due to non-ESBL-producing *K. pneumoniae* (Control Kp) and Patients with CO-UTI due to non-ESBL-producing *E. coli* (Control Ec).

Variable	Cases (n= 83)	Control Kp (n=155)	OR (95% CI)	p	Control Ec (n=164)	OR (95% CI)	p
Age, years (median, range)	78 (14 – 101)	79 (16 – 96)			78 (14 – 99)		
Gender, female	57 (68.7%)	108 (69.7%)			112 (68.3%)		
Functional Dependence*	24 (30.4%)	29 (21.8%)	2.15 (1 – 4.6)	0.050	25 (18%)	2.63 (1.2 – 5.76)	0.016
Health Care Relationship							
- Nursing home resident	17 (21.3%)	6 (4.1%)	5.67 (2.07 – 15.52)	0.001	8 (5%)	12.9 (2.9 – 56.75)	0.001
- Previous Hospital admission	13 (15.9%)	16 (10.3%)	1.93 (0.79 – 4.73)	0.15	10 (6.1%)	2.93 (1.2 – 7.15)	0.018
- True community-acquired	51 (63.8%)	127 (85.2%)	0.25 (0.11 - 0.53)	< 0.001	143 (88.8%)	0.1 (0.035 – 0.3)	< 0.001
Comorbidities							
- Charlson score (median, range)	1 (0 – 9)	1 (0 – 11)	1.09 (0.93 – 1.3)	0.28	1 (0 – 6)	1.24 (1.04 – 1.48)	0.016
- Recurrent UTI	39 (49.4%)	61 (41.2%)	1.57 (0.86 – 2.86)	0.14	54 (38.6%)	1.88 (0.99 – 3.58)	0.054
- Permanent urinary catheter	11 (13.9%)	14 (9%)	1.67 (0.68 – 4.14)	0.26	8 (5.2%)	3.41 (1.16 – 10.02)	0.026
Previous antibiotic treatment							
- Cephalosporins	27 (33.3%)	16 (11%)	4.06 (1.93 – 8.56)	< 0.001	22 (14.3%)	2.78 (1.43 – 5.41)	0.003
- Fluoroquinolones	22 (27.2%)	20 (13.7%)	2.66 (1.24 – 5.72)	0.012	27 (17.5%)	1.91 (0.94 – 3.87)	0.075
- Other antibiotic	14 (17.3%)	28 (19.2%)	0.95 (0.45 – 2)	0.9	19 (12.3%)	1.4 (0.69 – 2.83)	0.35

Note: Data are no. (%) of patients. CI, confidence interval; OR, odds ratio.

* Functional dependence defined as Barthel Index < 60.

Table 2. Multivariate Analysis of Risk Factors for CO-UTI due to ESBL-producing *K. pneumoniae*.

	Odds Ratio (95% CI)	p
Control non-ESBL-Kp		
Nursing home resident	8.8 (2.63 – 29.35)	0.000
Previous use of cephalosporins	4.01 (1.75 – 9.17)	0.001
Previous use of quinolones	3.27 (1.36 – 7.91)	0.008
Control non-ESBL-Ec		
Nursing home resident	9.58 (2.07 – 44.25)	0.004
Previous use of cephalosporins	2.49 (1.16 – 5.35)	0.019
Previous use of quinolones	2.11 (0.89 – 4.96)	0.089
Charlson Score	1.28 (1.03 – 1.59)	0.027

Table 3. Antibiotic resistance patterns of cases and *K. pneumoniae* control.

	Cases (n=83) (%)	Control-Kp (n=155) (%)	p
AMC	67 (80.7)	17 (11)	< 0.001
CTX	80 (96.4)	3 (1.9)	< 0.001
CAZ	71 (85.5)	3 (1.9)	< 0.001
CIP	60 (72.3)	15 (9.7)	< 0.001
SXT	60 (72.3)	15 (9.7)	< 0.001
FOSF	19 (22.9)	30 (19.4)	NS
GEN	35 (42.2)	3 (1.9)	< 0.001
NITRO	31 (37.3)	22 (14.2)	< 0.001
PTZ	27 (32.5)	3 (1.9)	< 0.001
Multiresistant strains	72 (86.7)	12 (7.7)	< 0.001

Note: figures represent numbers of resistant isolates (% Resistance).

AMC, amoxicillin/clavulanate; CTX, cefotaxime; CAZ,cefazolin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; FOSF, fosfomycin; GEN, gentamicin; NITR, nitrofurantoin; PTZ, piperacillin/tazobactam.

Supplementary Table S1. Checklist of items according to STROBE document.

	Recommendation	Assessment in article
Title and abstract	*a*) Indicate the study design with a commonly used term in the title or abstract *b*) Provide an informative and balanced summary in the abstract of what was done and what was found	a) Study design specified in title and abstract b) Balanced summary included in the abstract
Background/ rationale	Explain the scientific background and rationale for the investigation being reported	The scientific background and rationale are included in the introduction
Objectives	State specific objectives, including any pre-specified hypotheses	Objectives are stated in the introduction
Study design	Present key elements of study design early in the paper	Study design described in the first part of Methods
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Described in Methods
Participants	(*a*) Give the eligibility criteria and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	a) Described in Methods b) Matching criteria and number of cases and controls specified in Methods
Variables	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable	Defined in Methods
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Specified in Methods. The same methods for data collection of data were used in case and control groups.

Bias	Describe any efforts to address potential sources of bias	Matching controls by sex, age and year of isolation
Study size	Explain how the study size was arrived at	The basis of sample size is specified in Methods
Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Quantitative variables were handled as such. No groupings were made
Statistical methods	(*a*) Describe all statistical methods, including those used to control for confounding *b*) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	a) Included in Methods b) Included in Methods c) Patients with missing data were excluded d) No patient was lost to follow-up e) Not done
Participants *	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, (b) Give reasons for non-participation at each stage. (c) Consider use of a flow diagram	a) Described in methods b) Not done c) Not done
Descriptive data *	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders b) Indicate number of participants with missing data for each variable of interest	a), b) Described in Results and Table 1

Outcome data *	Report numbers of outcome events or summary measures over time	Table 1, 2 and 3
Main results *	*(a)* Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized *c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	a), b), c) Table 2 and 3
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	No interactions were found. No sensitivity analyses or subgroup analyses were performed.
Key results	Summarise key results with reference to study objectives	Specified in Abstract and Discussion
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Included in Discussion
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Included in Discussion
Generalisability	Discuss the generalisability (external validity) of the study results	Included in Discussion

Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Included

*Information is given separately for cases and controls.

SEGON ARTICLE*:*

High clonal diversity of ESBL-producing *Klebsiella pneumoniae* isolates from clinical samples in a non-outbreak situation. A cohort study.

Mariona Xercavins, Elena Jiménez, Emma Padilla, Montserrat Riera, Núria Freixas, Lucía Boix-Palop, Josefa Pérez and Esther Calbo.

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La doctoranda ha dissenyat l'estudi junt amb la directora de tesi, ha dut a terme junt amb altres autores els estudis microbiològics, la recollida de dades, l'anàlisi dels resultats i la redacció de l'article.

HIGH CLONAL DIVERSITY OF ESBL-PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES FROM CLINICAL SAMPLES IN A NON-OUTBREAK SITUATION. A COHORT STUDY.

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ABSTRACT

Background

Klebsiella pneumoniae has been responsible for a large number of clonal hospital outbreaks. However, some epidemiological changes have been observed since the emergence of CTX-M enzymes in *K. pneumoniae*.

Aim

To analyse the transmission dynamics of Extended Spectrum β-Lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp) in an acute care hospital.

Methods

In 2015 a prospective cohort study was conducted. All new consecutive adult patients with ESBL-Kp isolates in all clinical samples were included. Patients with a previous known infection/colonization by ESBL-Kp and patients in high risk areas (e.g., intensive care units) were excluded. Cross-transmission was defined as the carriage of a clonally-related ESBL-Kp between newly diagnosed patients who shared the same ward for ≥48 hours with another case, within a maximum time window of four weeks. ESBL-production was confirmed using the double-disk diffusion method and PCR. Clonal relationships were investigated by rep-PCR and multilocus sequence typing (MLST).

Results

Sixty *ESBL-Kp* isolates from 60 patients were included and analysed. Infections and colonizations were classified as hospital-acquired (52%), healthcare-related (40%) or community-acquired (8%).

High genetic diversity was detected. When epidemiological clinical data were combined with the rep-PCR, the patterns identified did not show any cases of cross-transmission. ESBL-Kp were detected in 12.5% of environmental samples. No clonal relationship could be established between environmental reservoirs and patients. The genetic mechanism detected in all strains was associated with *bla* $_{\text{CTX-M}}$ genes, and 97% were CTX-M-15.

Conclusions

The dynamics of ESBL-K. pneumoniae isolated in our setting could not be explained by clonal transmission from an index patient. A polyclonal spread of ESBL-Kp was identified.

Key words

ESBL; Klebsiella *pneumoniae*; clonal diversity.

INTRODUCTION

The epidemiology of healthcare-related infections has been characterized in recent decades by the emergence of Gram-negative multidrug-resistant organisms [1]. This increase in resistance appears to be due largely to the production of extended-spectrum β-lactamases (ESBLs) among all Enterobacterales. ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) is one of the most frequently identified multiresistant pathogens.

K. pneumoniae has been responsible for a large number of hospital outbreaks. In the 1990s, these outbreaks were clonal epidemics affecting mainly intensive care patients, and were due to SHV [2] and TEM enzyme types [3]. The first reports of CTX-M *K. pneumoniae* outbreaks were published in the 2000s [4]. Conversely, these CTX-M outbreaks were widespread in general hospital wards and their mortality rates are lower than those previously associated with SHV and TEM outbreaks.

Since the emergence of CTX-M β-lactamases, several clones harboring CTX-M-15 enzymes, often associated with other ESBL types, have been identified [5]. In the case of *K. pneumoniae* it seems that resistance is not restricted to a few genetic backgrounds, and that it is a phenomenon of multiple emergence rather than one involving the spread of a few clones [6]. In fact, high clonal diversity has been reported in non-outbreak [7] and outbreak [8] situations.

In the last five years, we have detected an increase in the incidence of hospital-acquired ESBL-Kp infections in our area (from 0.06 in 2011 to 0.35/1.000 stays in 2015) alongside a rise in the prevalence of community-acquired urinary tract infection due to ESBL-Kp (from 2.4% in 2010 to 10.3% in 2014). Most of these clinical isolates harbored CTX M-15 enzymes [9].

With the increase in ESBL-Kp among community-acquired cases [10,11] and in the hospital setting [4,12–14] there is a clear need to understand the dynamics of transmission of this relevant pathogen. It is crucial to determine whether the isolation of ESBL-Kp 48 hours after

hospital admission is actually caused by hospital cross-transmission, and also the extent to which it is preventable and merits infection control interventions.

In this scenario, the aim of the present study was to investigate the dynamics of transmission of ESBL-producing *Klebsiella pneumoniae* by assessing both the clinical epidemiological data and the clonal relatedness of ESBL-Kp among inpatients at a single academic acute care hospital.

MATERIAL AND METHODS

Setting and study design

In 2015 a prospective cohort study was conducted at Hospital Universitari Mútua Terrassa, Barcelona, a 400-bed acute care hospital with an annual mean number of 97,524 hospital stays for a population of 350,000 inhabitants. Patients are hosted in single or double rooms. Bathrooms are shared in double rooms.

Inclusion criteria

All new consecutive adult patients with ESBL-producing *Klebsiella pneumoniae* isolates from any specimens obtained by routine clinical practice were included in the study (one sample/patient). Patients with previous known infection/colonization by ESBL-Kp were excluded, as were adult patients admitted to intensive care units (ICU).

Clinical epidemiological data collection and infection control standards

Definitions:

- "Index patient" was defined as an inpatient with a newly recognized clinical sample yielding ESBL-Kp.
- "Contact patient" was defined as a person who shared the same room for ≥ 24 h with an index patient without initiation of contact precautions.
- "Cross-transmission" was defined as the carriage of a clonally-related ESBL-Kp among newly diagnosed patients sharing the same room or ward for ≥48 hours with another index case, within a maximum time window of four weeks.
- "Healthcare relation" was defined according to *Friedman et al*.[15] "Hospital-acquired infection" was defined as an infection acquired during hospital care that was not present or incubating at admission (infections occurring 48 h after admission were considered) or in a patient discharged from hospital in the previous 30 days. "Healthcare-related infection" was diagnosed if the patient fulfilled at least one of the following criteria: (i) having resided in a nursing home or long-term care facility in the 30 days before the episode; (ii) hospitalized in an acute care hospital for ≥48 h in the 90 days before the episode; (iii) having attended a hospital or hemodialysis clinic or received intravenous therapy in the 30 days before the episode; and/or (iv) having received intravenous therapy, wound care, enteral nutrition or healthcare at home in the 30 days before the episode. Otherwise, the infection was classified/considered as community-acquired.

All index patients were placed on contact precautions. Index patients were screened at the time of first detection in order to determine colonization. Screening samples included a rectal swab and urine sample in patients with a Foley catheter. According to ESCMID guidelines [16], three or more repeatedly negative screening cultures over the course of one or two weeks in a patient who had not received antimicrobial therapy for several weeks were needed to consider that a

patient was decolonized and, therefore, that contact precaution measures could be suspended. No active decolonization policies were conducted. Contact precautions included a single-patient room and the use of gloves and gowns by healthcare workers.

Basic infection control standards included proper hand hygiene (as indicated in the WHO guidelines) [17]. In 2015, compliance with a hospital-wide project promoting hand hygiene was 64%. During the study period a stringent environmental cleaning process including twice-daily hospital cleaning with detergents, as well as enhanced terminal cleaning of rooms of targeted patients on contact precautions, was also conducted.

Infection control staff routinely visited all inpatients with colonization or infection due to ESBL-producing *Klebsiella pneumoniae*. Data on demographics, type of sample, healthcare-relatedness, time from admission until ESBL-Kp identification and movements around the hospital (including detailed information regarding rooms and wards occupied during the hospital stay) were prospectively collected as part of the standard epidemiological clinical work-up conducted by the infection control team. Rectal swab screening for contact patients was also performed as well. All patients with known ESBL carriage were screened whenever they were readmitted to the hospital as part of standard infection control practices. No other active surveillance was applied.

Environmental samples

A surveys of environmental colonization of ESBL-Kp were performed in some of the rooms occupied by ESBL-Kp colonized or infected patients during the year under study. Four samples were obtained in each surveyed room (tap of the sink, surface around the sink, bedpan and bedpan washer tap). [18]

Samples were obtained for culture by rubbing gauzes moistened with thioglycolate broth repeatedly over designated sites in the immediate vicinity of the patient environment and they were stored in screw-cap sterile containers with 10mL of thioglycolate broth. The containers were incubated for 24 hours at 37ºC and then inoculated onto ChromID ESBL (bioMérieux) [4,19]

Microbiological methods

Bacterial identification and susceptibility testing was performed using Vitek2 System (BioMérieux). EUCAST breakpoints were used for interpretation of the results. ESBL-production was confirmed using the double-disk diffusion-method. ESBL characterization was performed by commercial PCR (Check-MDR CT103XL, Hain).

The genetic relationship between all 60 ESBL-Kp isolates was determined by automated repetitive-sequence-based PCR using the Diversilab system (bioMérieux), following the manufacturer's recommendations. Rep-PCR fingerprinting profiles were compared and analyzed by Diversilab (version 3.6) software using Pearson correlation coefficient pairwise pattern matching and the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. The cutoff value for similarity in order to establish strain identity was 95%.

Multilocus sequence typing (MLST) was performed using seven conserved housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB* and *tonB*). [20] The protocol of the MLST procedure, including allelic type and sequence type (ST) assignment methods, is available from the MLST databases of the Pasteur Institute (Paris, France) http://bigsdb.pasteur.fr/klebsiella/klebsiella.html. The phylogenetic relationships between the different ST found in the study were established by Phyloviz (https://online.phyloviz.net/index) using the goeBURST algorithm.

RESULTS

During 2015, 60 consecutive index cases were identified. Demographic and clinical data of patients and isolates are shown in Table 1. In order of frequency, the origins of the clinical samples were urine (47, 78%), surgical wounds (six, 10%), blood (six, 10%) and respiratory samples (one, 2%). Thirty-two isolates were obtained from patients admitted to the hospital emergency department, 16 in surgical wards and 12 in medical wards.

New index cases were detected with a median frequency of 2.5 (range 0-6) patients per month and there were no outbreaks in any specific hospital area (Figure 1). Counting all *K. pneumoniae* isolates, the proportion of samples with ESBL-producing enzymes in our hospital in 2015 was 26.10%, compared with 27.52% in Catalonia as a whole [21].

Among the clinical samples, 47 (78.3%) were interpreted as infections and 13 (21.7%) as colonizations.

Hospital-acquired infection/colonization was demonstrated in 31 index cases (52%), healthcare-related infection/colonization in 24 (40%) patients, and community-acquired infection/colonization in five (8%). Among healthcare-related samples, 11 (17%) were from nursing home residents. At two particular nursing homes two cases were identified, but neither a temporal nor a genetic relationship could be established between the isolates in either setting.

High genetic diversity was detected. The isolates were classified into 36 different patterns (rep-PCR, Diversilab); only 4/36 patterns included three or more isolates. Sixteen sequence types (ST) were identified. The most prevalent STs encountered were ST170 (23%), ST405 (21%) and ST392 (16%). Altogether, these STs represented 60% of the isolates. A summary of the characterization of the 60 isolates is shown in figure 2, and the phylogenetic relationships between the different STs are shown in figure 3.

Thirty-one strains with 19 different rep-PCR patterns and eight different STs were identified among hospital-acquired infection samples. The most frequent were ST170 (26%), ST405 (26%) and ST392 (23%).

Among healthcare-related cases, 24 strains were collected with 22 different patterns identified by rep-PCR and 11 STs. In this case the most frequent were ST170 (25%), ST405 (21%) and ST392 (13%).

Five community-acquired samples were included which showed five different patterns by rep-PCR and MLST. Two community-acquired sequence types (ST70 and ST307) were also found in two healthcare-related strains and in three hospital-acquired infection strains.

No cases of cross-transmission were found when epidemiological clinical data were combined with the rep-PCR patterns identified (figure 1).

The genetic mechanism detected in all ESBL-Kp isolates was associated with the presence of *bla* $_{CTX\text{-}M}$ genes. Most of the CTX-M detected belonged to group 1: CTX-M-15 (58 strains) and CTX-M-32 (one strain). One strain belonged to group 9. No carbapenemase producers were detected. All ESBL enzymes in hospital-acquired and community-acquired samples were CTX-M-15, as were 22 out of 24 identified in healthcare-related strains (the exceptions being one CTX-M-9 and one CTX-M-32).

Antibiotic susceptibility testing showed that all isolates were resistant to cefotaxime and ceftazidime. The antimicrobial resistance pattern is summarized in table 2. The phenotypical characterization showed differences between isolates with the same sequence type.

Environmental samples isolated

ESBL-Kp were detected in 4/32 (12.5%) environmental samples from three rooms.

One room had two positive samples (surface around the sink and bedpan washer tap) with an identical rep-PCR pattern, though it was not identified with a particular patient.

The other positive samples were isolated in two different rooms. The first one was on the surface around the sink with the same rep-PCR pattern as the strain isolated in the previous occupant of the room; the second was cultured from a sink tap and presented a rep-PCR pattern different from the one identified in a patient who had previously occupied the room.

DISCUSSION

In a non-outbreak setting, no cases of cross-transmission of ESBL-producing *Klebsiella pneumoniae* could be demonstrated in general wards (non-ICU) in our hospital during the year under study. A high genetic diversity was confirmed by both rep-PCR and MLST.

Traditionally, ESBL-*Kp* cross-transmission via the hands of healthcare workers [2] and the lower gastrointestinal tract of colonized patients [22] has been documented as the main reservoir of these microorganisms during hospital outbreaks [5,23]. However, in our setting, we could not demonstrate either a clonal or a clinical epidemiological relatedness between consecutive non-duplicate ESBL-*Kp* strains isolated during 2015. Therefore, this traditional dynamics cannot explain our epidemiology. A low rate of hospital-acquired infection transmitted by highly drug-resistant Gram-negative bacteria was also found in a large multicenter trial involving 18 Dutch hospitals, and in a single-center Swiss study of ESBL-producing Enterobacterales [24,25].

Interestingly, a recent study showed that only half of the cases of healthcare-acquired infection or colonization due to multi-drug resistant organisms (MDRO) according to CDC definitions are truly hospital-acquired [26]. Similarly, some reports suggest that some patients with infections caused by ESBL-producing K. pneumoniae isolates seen at hospitals should be epidemiologically defined as community-associated [9,11]. Therefore, in these scenarios, ESBL-producing K. pneumoniae is more likely to have been imported into the hospital than to have originated there. In fact, hospital outbreaks originating from a community source of ESBL-producing K. pneumoniae have already been described [4].

However a word of caution is in order before concluding that there is no transmission. Carriership is generally asymptomatic and universal screening is not conducted at our hospital. As a result, intermediate patients may be missed and no epidemiological link can be made. In addition, a seasonal variation was identified in our study. Nevertheless, it was recently shown that in a non-outbreak setting, importation of ESBL-producers into hospitals seems to be at least as frequent as transmission events during the hospital stay [27]. The total lack of clonal relatedness between index cases and contact patients makes cross-transmission highly improbable in our setting. It should also be stressed that for financial reasons universal screening for all MDRO is not carried out at most acute care hospitals.

Hospital environmental contamination has been reported as the source of several ESBL-Kp outbreaks [4,28,29]. In our study, no clonal relationship with environmental samples could be established. However, it is conceivable that more extensive environmental screening would have identified a reservoir possibly missed by the present design.

Only cross-transmitted MDRO are preventable and are reasonable targets for an infection control program. Non-preventable events may be related to selective antibiotic pressures that

trigger the emergence of ESBL-producing *K.pneumoniae* colonizing the gastrointestinal tract after admission. In this scenario, infection control measures must be coordinated with antimicrobial stewardship programs to stop the endemic evolution of ESBL-producing *K. pneumoniae*.

Regarding the antibiotic resistance phenotype, isolates were mostly classified as CTX-M 15 producers. These results are in agreement with those previously reported in other countries and thus corroborate the wide distribution of this enzyme [12,30–33]

The STs identified in our setting belong to previously described international clones associated with multidrug-resistant *K. pneumoniae* isolates. ST170 was the most frequent sequence type identified in our cohort. To our knowledge, this is the first report of ST170 in human strains. Moreover, ST170 was only detected in hospital strains without any epidemiological relationship, suggesting the possibility of an endemic situation. The other two frequently identified STs in our cohort (ST405 and ST392) have been described elsewhere in Europe and in South America in strains of human origin.

Machuca et al. [34] published the first report of a *K. pneumoniae* ST405 without harbouring a carbapenemase, the type we found in our isolates. Previously, ST405 has been described as a clone capable of disseminating different quinolone and beta-lactam resistance determinants (including ESBL and carbapenemase): in Spain and France among OXA-48 and CTX-M15-producing isolates, and in Yemen among NDM and CTX-M-15 producers. ST392 has been reported in *K. pneumoniae* in CTX-M-15 associated with carbapenemase: in KPC in China, and in OXA-48 in Europe. This is the first time that ST392 has been described in *K. pneumoniae* carrying only CTX-M.

The phenotypic method, consisting in the identification of species type and resistance towards several selected antibiotics, was unable to detect ST or rep-PCR groups. This suggests that the phenotypic method is not suitable for infection control procedures and that molecular identification is crucial for the definition of cross-transmission. Similar results were published by Souverein et al. [8].

This study has some limitations. First, the single-center study design may limit the generalizability to other settings and we cannot rule out the possibility that the lack of transmission at our institution may be attributable to the high level of infection control and to the low number of beds per room.

Second, no plasmid typing was performed. The criteria for cross-transmission in the present study did not address the possibility of horizontal transmission of common plasmids between different Enterobacterales species [6,35].

Third, the gold standard assay for molecular typing is pulsed-field gel electrophoresis (PFGE), due to its high discriminatory power. The discriminatory power of rep-PCR is generally similar to that of PFGE. PCR methods are preferable in the study of small, time-limited outbreaks, while in complex outbreaks of longer duration, in which clonal evolution and dynamics are studied, PFGE should be used. Molecular typing methods based on DNA sequencing such as MLST are applicable in global epidemiological studies [36]. The initial assessment in our study was made using the rep-PCR, and the MLST method confirmed the diversity in our population.

Fourth, the lack of systematic active surveillance of all inpatients admitted or discharged from hospital may have meant that some transmission events were missed. However, systematic surveillance of all contact patients did not demonstrate cross-transmission in this high-risk

situation. Fifth, the detection method, i.e., screening for colonization merely by collecting rectal swabs without any enrichment to increase the detection sensitivity, may have missed some ESBL-KP strains in contact patients. Finally, since we only studied ESBL-Kp in a non-outbreak scenario, it may not be possible to extrapolate our results to other Enterobacterales or to other epidemic settings.

In conclusion, in this epidemiological study of a non-outbreak setting, we identified a polyclonal spread of ESBL-Kp with high genetic diversity. Neither clonal cross-transmission nor environmental reservoirs could be demonstrated. Our data suggest that the probable importation of ESBL-Kp into the hospital may explain the dynamics of hospital-acquired cases in our setting. These findings question the validity of the contact precaution measures applied to control ESBL-Kp epidemics. More studies are now required to explore this matter further.

Abbreviations

CDC: Centers for Disease Control and Prevention; EUCAST: European Committee on antimicrobial susceptibility testing; ESBL: Extended-spectrum β-lactamases; ESBL-Kp: Extended-spectrum β-lactamases-Klebsiella pneumoniae; ESCMID: European Society for Clinical Microbiology and Infectious Diseases; ICU: Intensive care unit; MDRO: Multi-drug resistant organisms; MLST: Multilocus sequence typing; PCR: Polymerase chain reaction; ST: Sequence type; WHO: World Health Organization

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Availability of data and materials

Not applicable

Authors' contributions

MX and EC designed the study. MX, EJ, EP, JP were responsible for performing all laboratory tests. MX, MR, NF were responsible for data collection. MX, EJ, EP, LB, EC analysed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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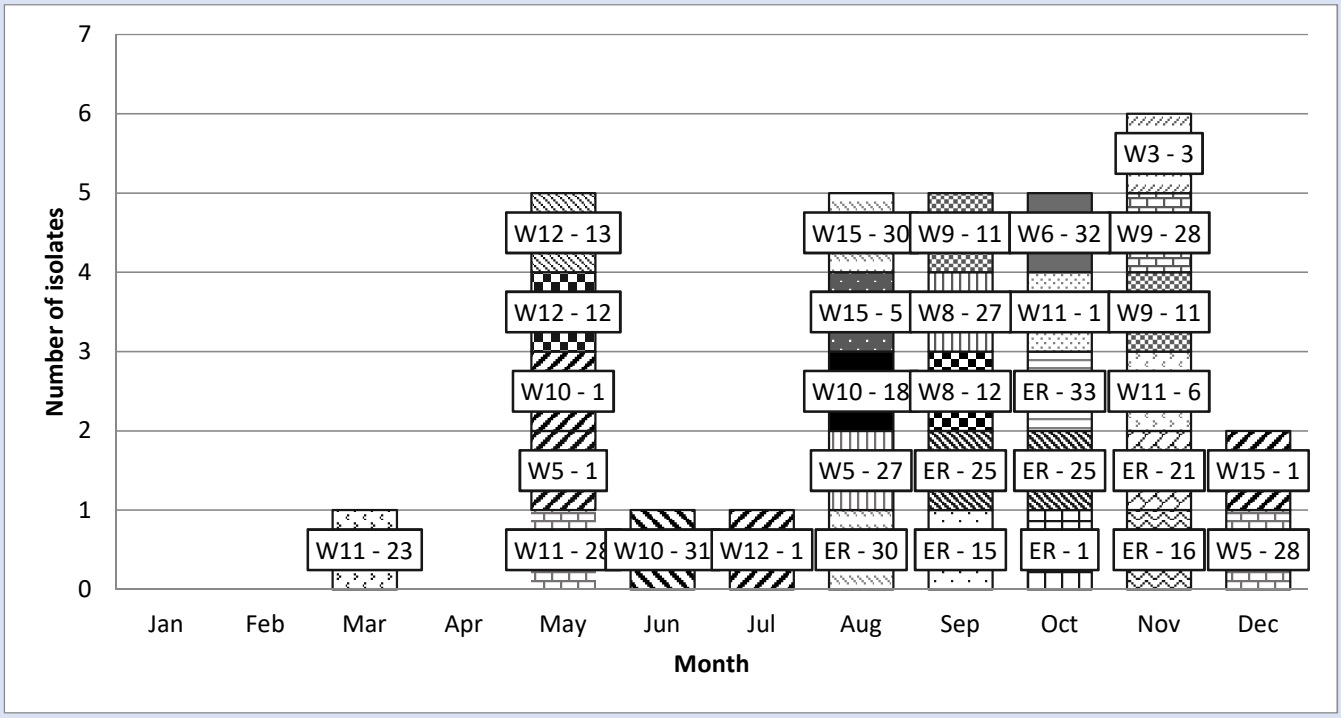
Table 1. Demographics and clinical characteristics of patients and isolates

Patients characteristics	Community-acquired	Healthcare-related	Hospital-acquired infection	Total
Number of isolates and patients	5	24	31	60
Gender, male (N)	1	12	20	33
Sample sites (N) - Urine - Wounds - Blood - Respiratory	 5 0 0 0	 21 1 2 0	 21 5 4 1	 47 6 6 1
Units (N) - Surgery wards - Medical wards - Emergency department	 1 4	 2 22	 13 12 6	 16 12 32

Table 2Antimicrobial resistance of *K. pneumoniae* ESBL-producing isolates

Antibiotic	Community-acquired (N=5)	Healthcare-related N=24	Hospital-adquired infection (N=31)	Total
Amoxicillin-clavulanic acid	3/5	21/24	31/31	55/60
Amikacin	0/5	2/24	1/27	3/57
Cefepime	5/5	24/24	31/31	60/60
Cefuroxime	5/5	24/24	31/31	60/60
Cefotaxime	5/5	24/24	31/31	60/60
Ceftazidime	5/5	24/24	31/31	60/60
Ciprofloxacin	3/5	21/24	31/31	55/60
Ertapenem	0/5	0/24	0/31	0 / 60
Gentamicin	1/5	13/24	14/31	28 /60
Imipenem	0/5	0/24	0 /31	0 /60
Piperacillin/tazobactam	2/5	12/24	20/31	34/60
Trimethoprim/sulfamethoxazole	3/5	21/24	29/31	53/60

Figure 1. rep-PCR pattern of ESBL-*K. pneumoniae* isolates of hospital-acquired infection origin per month and ward.



Each square represents the number of isolates; every rep-PCR pattern is represented by a different drawing. All the isolates are labelled according to the ward (W) or ER (Emergency room) and rep-PCR pattern (number).

Figure 2. Cluster analysis and virtual image from Diversilab generated fingerprints of the 60 *K. pneumoniae* strains, including corresponding ST results from MLST.

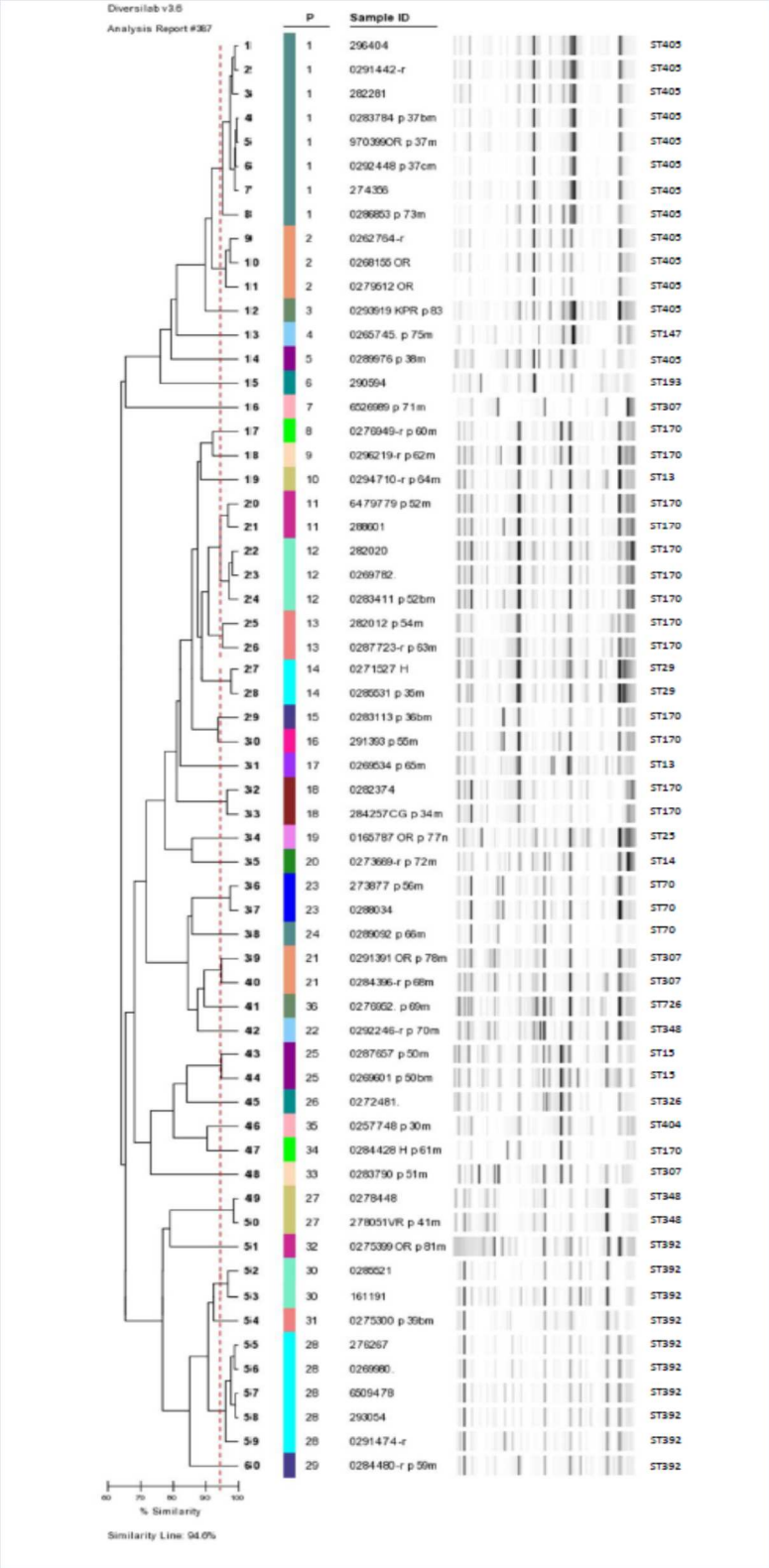
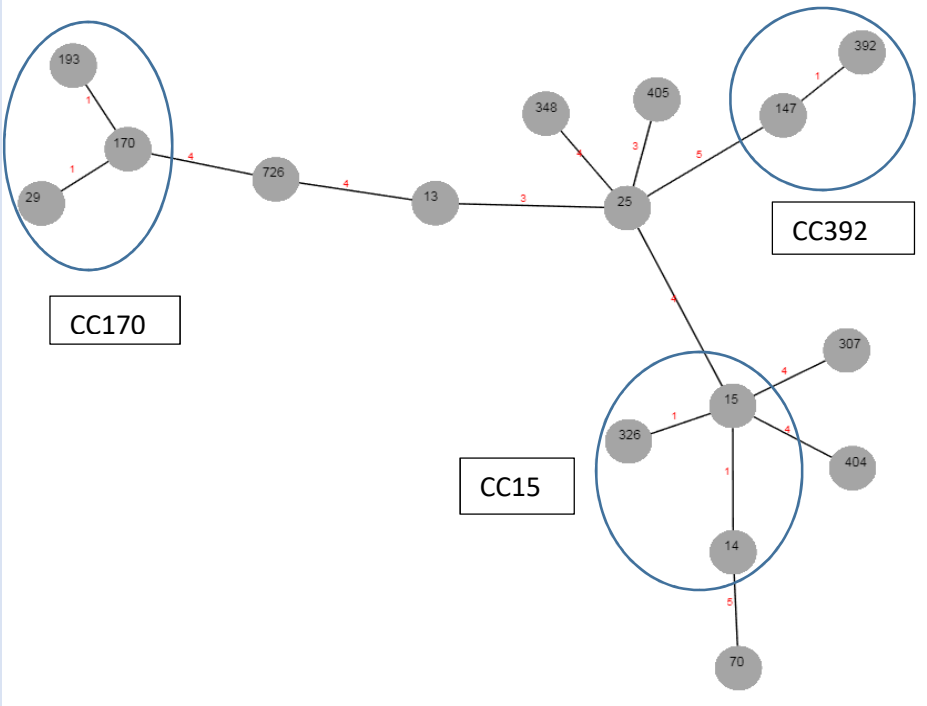


Figure 3. Phylogenetic relationships between the STs detected in our study.



Supplementary Table S1. Checklist of items according to STROBE document.

	Recommendation	Assessment in article
Title and abstract	*a*) Indicate the study design with a commonly used term in the title or abstract *b*) Provide an informative and balanced summary in the abstract of what was done and what was found	a) Study design specified in title and abstract b) Balanced summary included in the abstract
Background/ rationale	Explain the scientific background and rationale for the investigation being reported	The scientific background and rationale are included in the introduction
Objectives	State specific objectives, including any pre-specified hypotheses	Objectives are stated in the introduction
Study design	Present key elements of study design early in the paper	Study design described in the first part of Methods
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Described in Methods
Participants	Give the eligibility criteria and the sources and methods of selection of participants. Describe methods of follow-up	Described in Methods
Variables	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable	Defined in Methods
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement).	Specified in Methods.
Bias	Describe any efforts to address potential sources of bias	Local epidemiology can impact on the incidence of ST and rep-PCR analyzed.
Study size	Explain how the study size was arrived at	Sample size is specified in Methods: all cases during the study period

Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Quantitative variables were handled as such. No groupings were made
Statistical methods	(*a*) Describe all statistical methods, including those used to control for confounding *b*) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	f) Included in Methods g) Included in Methods h) Variables with missing data are specified in the analysis i) No patient was lost to follow-up j) Not done
Participants	(d) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, (e) Give reasons for non-participation at each stage. (f) Consider use of a flow diagram	a) Described in methods b) Not done c) Not done
Descriptive data	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders b) Indicate number of participants with missing data for each variable of interest	a), b) Described in Results and Table 2
Outcome data	Report numbers of outcome events or summary measures over time	Table 2, 3, 4 and 5
Main results	*(b)* Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	a), b) Table 2, 3 ad 4 c) Not done

	(*b*) Report category boundaries when continuous variables were categorized *c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.	
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses.	No interactions were found. No sensitivity analyses or subgroup analyses were performed.
Key results	Summarise key results with reference to study objectives	Specified in Abstract and Discussion
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Included in Discussion
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Included in Discussion
Generalisability	Discuss the generalisability (external validity) of the study results	Included in Discussion
Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Included

CHARACTERISTICS COMMUNITY-ACQUIRED ISOLATES

Isolate	Date	Specimen	β-Lactamase	rep-PCR	ST	Infection/Colonization
16	28/12/2015	Urine	CTXM-15	7	307	I
34	02/07/2015	Urine	CTXM-15	19	25	I
37	22/08/2015	Urine	CTXM-15	23	70	C
45	02/03/2015	Urine	CTXM-15	26	326	I
46	21/07/2015	Urine	CTXM-15	35	404	I

CHARACTERISTICS HEALTHCARE-RELATED ISOLATES

Isolate	Date	Specimen	β-Lactamase	rep-PCR	ST	Infection/Colonization
19	12/11/2015	Urine	CTXM-15	10	13	I
31	13/10/2015	Urine	CTXM-15	17	13	I
2	29/11/2015	Blood	CTXM-15	1	405	I
8	07/09/2015	Urine	CTXM-15	1	405	I
9	06/01/2015	Urine	CTXM-15	2	405	I
10	02/01/2015	Urine	CTXM-15	2	405	I
11	30/04/2015	Urine	CTXM-15	2	405	I
13	09/01/2015	Urine	CTX-M-32	4	147	I
17	02/05/2015	Urine	CTXM-15	8	170	C
18	20/12/2015	Urine	CTXM-15	9	170	I
23	06/07/2015	Urine	CTXM-15	12	170	I
26	20/09/2015	Urine	CTXM-15	13	170	I
28	06/08/2015	Urine	CTXM-15	14	29	I
27	28/05/2015	Blood	CTXM-15	14	29	I
33	21/08/2015	Urine	CTXM-15	18	170	C
35	30/06/2015	Urine	CTXM-15	20	14	I
40	28/08/2015	Urine	CTXM-15	21	307	C
42	25/10/2015	Urine	CTXM-15	22	348	C
38	18/08/2015	Urine	CTXM-15	24	70	C
59	02/12/2015	Urine	CTXM-15	28	392	I
56	10/06/2015	Urine	CTXM-15	28	392	I
60	27/08/2015	Urine	CTXM-15	29	392	I
47	15/08/2015	Urine	CTXM-15	34	170	I
41	02/05/2015	Surgical wound	CTX-M-9	36	726	C

CHARACTERISTICS HOSPITAL-ACQUIRED ISOLATES

Isolate	Date	Specimen	β-Lactamase	rep-PCR	ST	Infection/Colonization
3	22/05/2015	Surgical wound	CTXM-15	1	405	I
7	26/05/2015	Surgical wound	CTXM-15	1	405	I
1	15/12/2015	Urine	CTXM-15	1	405	I
5	31/07/2015	Urine	CTXM-15	1	405	I
4	05/10/2015	Blood	CTXM-15	1	405	I
6	07/10/2015	Urine	CTXM-15	1	405	I
12	18/11/2015	Surgical wound	CTXM-15	3	405	I
14	13/08/2015	Urine	CTXM-15	5	405	I
15	11/11/2015	Surgical wound	CTXM-15	6	193	C
20	24/09/2015	Urine	CTXM-15	11	170	C
21	18/11/2015	Urine	CTXM-15	11	170	C
22	15/05/2015	Urine	CTXM-15	12	170	I
24	22/09/2015	Blood	CTXM-15	12	170	I
25	03/05/2015	Urine	CTXM-15	13	170	I
29	23/09/2015	Urine	CTXM-15	15	170	I
30	23/11/2015	Urine	CTXM-15	16	170	I
32	15/08/2015	Urine	CTXM-15	18	170	I
39	22/11/2015	Urine	CTXM-15	21	307	I
36	18/03/2015	Blood	CTXM-15	23	70	I
43	27/09/2015	Urine	CTXM-15	25	15	I
44	06/10/2015	Urine	CTXM-15	25	15	I
50	03/08/2015	Respiratory	CTXM-15	27	348	I
49	30/09/2015	Urine	CTXM-15	27	348	I
55	15/05/2015	Urine	CTXM-15	28	392	C
57	19/11/2015	Urine	CTXM-15	28	392	C
58	21/12/2015	Urine	CTXM-15	28	392	I
52	02/08/2015	Urine	CTXM-15	30	392	C
53	27/08/2015	Surgical wound	CTXM-15	30	392	I
54	10/06/2015	Urine	CTXM-15	31	392	I
51	5/10/2015	Urine	CTXM-15	32	392	I
48	02/10/2015	Blood	CTXM-15	33	307	I

VII. DISCUSSIÓ

L'epidemiologia de les infeccions relacionades amb l'assistència sanitària s'ha caracteritzat a les últimes dècades per l'emergència de microorganismes gramnegatius multiresistents [126]. Aquest increment de resistències és degut en part a la producció de β-lactamases d'espectre estès pels Enterobacterales. *Klebsiella pneumoniae* productora de BLEE (KpBLEE) és un dels patògens multiresistents més freqüentment identificats.

KpBLEE és un microorganisme reconegut com a responsable d'infeccions d'adquisició hospitalària i d'un gran nombre de brots hospitalaris. Tradicionalment s'han descrit a unitats de cures intensives, a on el consum d'antibiòtics és més alt i la possible transmissió pacient a pacient és més factible [30,59,61,72,74,127]

Fins al final de la dècada del 1990, els aïllaments que expressen BLEEs eren principalment adquirits a l'hospital i quasi sempre deguts a tipus d' SHV [29] o TEM [30]. Amb l'emergència dels enzims CTX-M, a l'inici del segle XXI [26], ha canviat l'epidemiologia radicalment, els brots passen a estar disseminats per l'hospital (Taula 1) en comptes de quedar situats en una planta en concret i els seus percentatges de mortalitat són més baixos que els descrits prèviament als brots produïts per SHV o per TEM [26].

Existeixen dades que suggereixen que la presència de KpBLEE, i específicament, CTX-M-15, podrien estar emergint de la comunitat [26,33,128–132].

Aquesta situació és el resultat de la disseminació de clones epidèmiques de *Klebsiella pneumoniae* productora de CTX-M-15 i de diferents plasmidis portadors de mecanismes de resistència contra diferents antimicrobians, un fenomen que ha contribuït a la seva selecció [28].

Durant el període 2011-2015 a la nostra àrea vam detectar per una banda un increment de les infeccions per KpBLEE adquirides a l'hospital (de 0,06 el 2011 a 0,35 / 1.000 estades el 2015 i per l'altra banda un augment de la prevalença de les infeccions del tracte urinari degudes a KpBLEE d'origen comunitari, segons els criteris de Friedman [119].

Per aquests motius ens va interessar entendre l'epidemiologia de les KpBLEE a la nostra zona i per això s'ha plantejat el problema a través de dues vessants: les infeccions urinàries d'origen comunitari i l'estudi de totes les soques aïllades a un hospital general universitari durant un any.

En el primer objectiu, hem identificat un augment important de la prevalença de KpBLEE per CTX-M-15 causant ITU-Co a una àmplia àrea de salut: d'un 2,4% al 2010 a un 10,3% el 2014. Aquestes troballes són consistents amb publicacions confirmant l'augment de KpBLEE a la comunitat [33,95,128,129,133–135].

Destaca notablement que quasi les 2/3 parts d'aquests pacients no han estat hospitalitzats durant els 3 mesos previs, tampoc han tingut cap contacte amb el medi sanitari ni han estat ingressats en centres soci-sanitaris o residències, pel que veritablement es consideren infeccions d'origen comunitari, més del 75% de les soques són multiresistents i, la majoria resistent a fluorquinolones i cotrimoxazol.

Els resultats d'aquest primer estudi presenten un escenari preocupant a la comunitat: el segon agent etiològic més freqüent causant d'ITU-Co és ara també portador de CTX-M-15, un enzim associat inicialment a *E. coli*. Per altra banda, alguns pacients amb infeccions degudes a KpBLEE atesos a l'hospital són ara epidemiològicament considerades com a adquirides a la comunitat. Si aquestes troballes es confirmen a altres àrees, ens pot portar a un canvi de vista epidemiològic i de les polítiques de control d'infecció.

Per a investigar els factors de risc associats a les ITU-Co d'un microorganisme específic , el grup control s'ha de triar entre pacients amb bacteris sensibles de la mateixa espècie. Però aquest disseny pot sobreestimar la importància de l'ús previ d'antibiòtics perquè els pacients que han rebut antimicrobians seran probablement infra-representats en el grup control. Aquest problema es pot evitar escollint pacients controls entre tots els pacients a risc, malgrat alguns dels factors de risc identificats no es podran associar específicament amb el risc de desenvolupar una infecció per un microorganisme sensible [136]. Per aquesta raó, s'ha dissenyat un estudi cas-control-

control, similar al disseny que va utilitzar Rodríguez-Baño, et al [137] per tal d'identificar els factors de risc de les bacterièmies causades per *E. coli* productora de BLEE.

Els factors de risc per a ambdues poblacions han sigut el fet de residir en un centre soci-sanitari o residència i l'haver pres cefalosporines, indicant que aquests estan veritablement associats a ITU-Co per KpBLEE. L'ús previ de quinolones és un factor de risc específic per ITU-Co per KpBLEE en el grup control KpnoBLEE, però tenint en compte que no s'ha trobat en el grup control EcnoBLEE, l'associació pot sobreestimar la importància de l'ús previ de quinolones.

Els dos factors de risc identificats en aquest estudi són similars als reportats prèviament com a causa d'infeccions adquirides a la comunitat i causades per altres enterobacteris, principalment per *E. coli* productor de BLEE (EcBLEE) [19,138–140]. Però, fins ara, EcBLEE i KpBLEE han presentat diferències en la seva epidemiologia deguda a una dinàmica de transmissió diferent [116].

Totes aquestes dades fan pensar que l'adquisició de l'enzim CTX-M-15 per *K. pneumoniae* als aïllaments comunitaris podria ser com el fenomen descrit fa uns anys amb *E. coli,* suggerint que la interfase entre hospitals i comunitat pot quedar desdibuixada. Trobem molts exemples de la presència de KpBLEE a la comunitat:

- Portadors fecals en pacients comunitaris i en persones sanes a diferents àrees geogràfiques [24,78,80,81,86–89,96,141].
- Mostres clíniques de pacients de la comunitat [94,128].
- Aigua de l'aixeta [65], aigües residuals tractades [84] i aigües residuals hospitalàries [79].
- Menjar [31,78].
- Animals de companyia i salvatges [90–93,142].

Totes aquestes dades contribueixen a canviar el nostre tradicional punt de vista epidemiològic.

CTX-M-15 és l'enzim més freqüent identificat en el nostre estudi. Les CTX-M s'han estès epidèmicament entre els enterobacteris i ara són el tipus de BLEE més

dominant en els aïllaments clínics, principalment *E. coli* [143]. Un factor important de la seva dominància és l'amplia disseminació de clons bacterians produint tipus de CTX-M. De manera que la disseminació clonal d' *E. coli* CTX-M-15 del grup filogenètic B2 i ST 131 ha sigut identificat com el més prevalent en molts països [84]. No s'ha detectat disseminació clonal en la cohort del nostre estudi, fet que també descriu Pons, *et al* [94], contràriament a les epidèmies per EcBLEE descrites anteriorment [144–147]. Probablement, la ràpida emergència d' *E. coli* CTX-M-15 ha generat un gran reservori genètic , del qual altres espècies, com *K. pneumoniae,* han pogut adquirir fàcilment aquest gen de resistència [24]. Però amb *K. pneumoniae* sembla que la resistència és un fenomen de múltiple emergència més que la disseminació d'uns pocs clons [148].

Aquest estudi té algunes limitacions. Les dades estan recollides retrospectivament de les històries clíniques de l'hospital i de l'assistència primària, fet que ha provocat que a vegades sigui difícil saber amb precisió la síndrome clínica per manca d'informació. No s'han inclòs els pacients d'urgències, els qual haurien pogut contribuir a una infra representació dels casos adquirits a la comunitat. A l'estudi cas-control-control l'aparellament es va fer per sexe i edat per tal d'evitar confusions amb co-morbilitats i l'alta prevalença de la UTI baixa en dones, respectivament. Per això, aquestes variables no s'han estudiat com a factors de risc. Solament una tercera part de les soques han estat disponibles per a fer la caracterització enzimàtica i l'anàlisi dels plasmidis no s'ha realitzat. Finalment, l'estudi s'ha realitzat en un únic hospital universitari que dóna assistència a una població adscrita a una regió geogràfica, fet que fa que els resultats no poden ser extrapolables a altres centres.

Malgrat les limitacions, l'estudi de l'epidemiologia i factors de risc associats a les infeccions del tracte urinari d'origen comunitari produïdes per KpBLEE, mostra un increment en la prevalença d'aquestes infeccions. L' exposició prèvia a cefalosporines així com ser resident d'un centre sanitari o residència estan clarament associats amb l'aïllament de KpBLEE. L'enzim CTX-M-15 reemplaça altres tipus de BLEEs i passa a ser l'enzim predominant en diferents clones de *K. pneumoniae* [24].

Els resultats d'aquest primer estudi poden tenir algunes conseqüències pràctiques. Primer, s'hauria de fer accions per tal de reduir l'ús de cefalosporines,

sempre que sigui possible, com a tractament de les ITU. Segon quan es dissenyin protocols de tractament empíric cal tenir en compte el considerar antimicrobians actius contra microorganismes productors de BLEEs a pacients amb sèpsia greu urinària d'origen comunitari d'alt risc. Tercer, les mesures de control d'infecció hospitalària hauran de contemplar aquesta epidemiologia de *K. pneumoniae* productora de CTX-M-15 i adaptar les mesures de prevenció a l'adquisició comunitària no prevenible des de l'àmbit d'acció dels equips de control d'infecció.

En el segon objectiu d'aquesta tesi , hem descrit que a l'Hospital Universitari Mútua Terrassa durant l'any 2015 en una situació de no brot, no es va demostrar cap cas de transmissió creuada a plantes d'hospitalització general (no unitats de cures intensives) i es va confirmar una gran diversitat genètica mitjançant tècniques de rep-PCR i MLST.

Amb l'increment de KpBLEE entre els casos comunitaris [128,149] i en els centres hospitalaris [24,31,60,67] és necessari entendre la dinàmica de transmissió en un hospital, doncs és crucial saber si l'aïllament d'una soca de KpBLEE 48 hores després d'un ingrés hospitalari és deguda o no a una transmissió creuada [113].

Tradicionalment, la transmissió creuada de KpBLEE és mitjançant les mans del personal sanitari [29,150] i el tracte intestinal inferior dels pacients colonitzats [151] també ha estat documentat com a reservori principal d'aquests microorganismes durant els brots hospitalaris [26,150]. No obstant, en el nostre centre, no hem pogut demostrar tampoc una relació ni clínic- epidemiològica ni clonal en 60 soques consecutives, no duplicades de KpBLEE aïllades durant l'any 2015. Per tant, aquesta dinàmica tradicional no pot explicar la nostra epidemiologia. De forma similar, coincideix amb les descripcions fetes per altres autors: a un estudi multicèntric a 18 hospitals holandesos [152] i a un altre d'un centre suís [153] també es troba una taxa baixa de transmissió nosocomial d' Enterobacterales productors de BLEE.

Curiosament, un estudi recent mostra que només la mitat dels casos d'infeccions o colonitzacions relacionades amb l'assistència sanitària degudes a microorganismes multiresistents (MMR) [154], d'acord amb les definicions del CDC, són veritablement d'adquisició hospitalària [155]. De la mateixa manera, altres estudis, a part del nostre

sobre infeccions urinàries, suggereixen que pacients amb infeccions per KpBLEE diagnosticades a l'hospital tenen epidemiològicament un origen comunitari [128]. En aquest escenari, és més probable que les KpBLEE hagin estat importades a l'hospital que haver-se originat en ell. De fet, s'han descrits brots hospitalaris amb una font comunitària [31,65].

Però cal ser molt prudents en concloure que no hi ha transmissió. Els portadors són generalment asimptomàtics i en el nostre hospital no fem una recerca activa universal per a MMR. Això fa que, pacients intermedis es puguin escapar i no es pugui dur a terme la vinculació epidemiològica. No obstant, s'ha demostrat que en un centre sense brots, la importació dins de l'hospital de microorganismes productors de BLEE és tan freqüent com la transmissió durant l'estada hospitalària [156]. La falta total de relació clonal entre els casos índex i els pacients contacte fa que la transmissió creuada sigui altament improbable en el nostre centre hospitalari. També cal destacar, que per raons econòmiques i organitzatives, no s'instaura la recerca activa de portadors universal de MMR a la majoria d'hospitals d'aguts.

La contaminació de l'ambient hospitalari ha estat descrita com a font de diferents brots per KpBLEE [31,123,157–159]. En el nostre estudi, però, no hem pogut identificar un reservori ni establir cap relació clonal.

Les infeccions causades per MMR d'adquisició comunitària i debut hospitalari no poden ser evitades per les polítiques de prevenció i control de la infecció. Tan sols la transmissió creuada de MMR pot ser evitada i aquest és un dels objectius principals d'un equip de Control d'Infecció [160]. Però hi ha esdeveniments no evitables que es poden relacionar amb la pressió antibiòtica, la qual desencadena l'emergència de la KpBLEE com a colonitzant del tracte gastrointestinal després de l'admissió. En aquest escenari és bàsic que les mesures de control d'infecció es coordinin amb els programes d'optimització de l'ús d'antibiòtics per tal d'obtindre un efecte sinèrgic i així reduir la disseminació de MMR com la KpBLEE [161].

Pel que fa al tipus d'enzim de resistència antibiòtica, la majoria van ser classificats com a CTX-M-15. Aquests resultats estan d'acord amb resultats prèviament publicats a

altres països que corroboren l'amplia distribució mundial d'aquest enzim [24,69,129,162–164].

Els mètodes de tipificació de les soques bacterianes són bàsics per a estudiar la transmissió [113]. Els STs identificats en el nostre hospital pertanyen a clones prèviament descrites i associades amb soques de *K. pneumoniae* multiresistent. ST170 és el *seqüenciotip* més freqüentment identificat a la nostra cohort. Pel coneixement que tenim fins ara, aquest és la primera descripció d' ST170 a soques humanes. A més, ST70 sols s'ha detectat a soques hospitalàries sense cap relació epidemiològica entre elles, suggerint la possibilitat d'una situació endèmica. Els altres dos STs més freqüents de la nostra cohort (ST406 i ST392) han estat descrits a soques d'origen humà a altres llocs d'Europa i Amèrica del Sud.

Machuca, et al [165] han publicat la primera descripció de *K. pneumoniae* ST405 productora de BLEE i no portadora de carbapenemasa, que és el tipus que hem trobat al nostre estudi. Prèviament, s'ha estat descrit com un clon capaç de disseminar diferents determinants de resistència a quinolones i β-lactàmics (incloent BLEE i carbapenemasa): a Espanya i França entre els aïllaments productors de CTX-M-15 i OXA-48, i al Iemen a productors de CTX-M15 i NDM. ST 392 ja ha estat descrit en KpBLEE CTX-M-15 associats amb carbapenemases: amb KPC a la Xina i amb OXA-48 a Europa. Aquesta és la primera vegada que l'ST392 ha estat descrit a *K. pneumoniae* portadora sols de CTX-M.

La manca de clonalitat de les soques de l'estudi tant pel mètode de rep-PCR com per la heterogeneïtat dels STs mostra la gran variabilitat en el nostre medi, fet també descrit a altres zones [33,94,132].

Els mètodes fenotípics, com l'antibiograma, no han estat capaços de detectar grups de rep-PCR o grups d' ST, per tant, la identificació molecular és fonamental per a definir si hi ha o no transmissió creuada. Resultats similars estan publicats per Souverein, et al [32]. Actualment la seqüenciació total del genoma és una eina útil per a analitzar, confirmar i comprendre millor els brots [166], però el seu ús durant un brot nosocomial té en aquests moments limitacions en quant a cost, disponibilitat i personal qualificat.

Ara bé, tècniques de PCR senzilles, però fiables i específiques, basades en les dades de la seqüenciació massiva, podrien ser la solució a les limitacions esmentades [114].

L'estudi té algunes limitacions. El disseny fet en un únic centre hospitalari limita la generalització a altres centres i no podem descartar la possibilitat que la no transmissió a la nostra institució pugui ser atribuïda a l'alt nivell de control d'infecció i al nombre baix de llits per habitació. No s'ha realitzat estudi de plasmidis, doncs en el criteri de transmissió creuada no es va tenir en compte la possibilitat de transmissió horitzontal entre diferents espècies d'enterobacteris [148]. El mètode molecular "*Gold Standard*" és l'electroforesi en camp polsant pel seu gran poder discriminatori. El poder discriminatori de la rep-PCR és, generalment, similar a l'ECP. Els mètodes mitjançant PCR són preferibles en l'estudi d'un nombre reduït de soques o en brots petits. Els mètodes de tipificació basats en la seqüenciació de l'ADN, col el MLST, són útils en estudis d'epidemiologia global [105]. La manca de recerca activa sistemàtica de MMR a tots els pacients ingressats o dels provinents d'un altre hospital pot haver contribuït a no conèixer casos de portadors asimptomàtics intermediaris que podrien ser causa d'una possible transmissió [32]. També el fet de no utilitzar un medi d'enriquiment a partir dels escovillons rectals pot haver disminuït la recuperació d'algunes soques de KpBLEE [167,168]. Finalment, el fet de sols estudiar la KpBLEE en un escenari de no brot fa que no sigui possible extrapolar els nostres resultats a altres enterobacteris o a altres ambients epidèmics.

Els resultats d'aquests dos estudis realitzats ens han ajudat a entendre millor l'epidemiologia de la KpBLEE al nostre àmbit. Hem constatat que a part de ser d'adquisició hospitalària o relacionada amb l'assistència sanitària també pot tenir el seu origen a la comunitat i causar patologia.

Aquestes dades ens podran ajudar, tant, a la racionalització de les polítiques de control d'infecció durant l'ingrés hospitalari, valorant en quins pacients amb KpBLEE i a quines àrees de l'hospital caldria realitzar precaucions de contacte o seria suficient amb precaucions estàndard [169–171], així com a realitzar el disseny dels tractaments

empírics en funció dels factors de risc de KpBLEE per tal d'optimitzar l'ús d'antibiòtics [172,173].

El fet de que existeixen múltiples nivells de transmissió de soques de KpBLEE, de plasmidis i de gens, així com també diferents reservoris (centres hospitalaris, centres soci-sanitaris, productes alimentaris, aigua, animals salvatges i de companyia), fa que es necessiti un plantejament global d'actuació, el qual queda inclòs en el projecte "One-Health" de l' Organització Mundial de la Salut [174].

Aquest projecte està concebut per a treballar per a la protecció de la salut pública mitjançant polítiques de prevenció i control de patògens actuant a tres nivells simultàniament: humans, animals i medi ambient.

Estudis posteriors ens aniran ajudant a assolir tots aquests reptes.

VIII. CONCLUSIONS

1. La prevalença de les infeccions urinàries d'origen comunitari degudes a *K. pneumoniae* productora de BLEE està augmentant. Destaca, que quasi en els 2/3 de casos són veritablement comunitàries.

2. Els factors de risc identificats per a ITU-Co causada per KpBLEE són l'exposició a les cefalosporines i ser resident d'un centre sociosanitari o residència.

3. A les infeccions del tracte urinari els enzims CTX-M-15 són els més freqüents i estan reemplaçant tipus més antics de BLEE a *K. pneumoniae*.

4. A l'estudi epidemiològic d'un centre hospitalari d'aguts en situació de no brot hem identificat una disseminació policlonal de KpBLEE amb alta diversitat genètica, sense poder demostrar ni transmissió creuada ni reservoris ambientals dins de l'hospital.

5. La β-lactamasa CTX-M-15 és la més freqüent (96.7%) a les soques KpBLEE hospitalàries.

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X. ANNEXES

PRESENTACIONS A CONGRESSOS:

- L. Boix-Palop, J. Pérez, C. Badía, **M. Xercavins**, M. Obradors, M. Rodríguez, M. Simó, J. Garau, E. Calbo. *Klebsiella pneumoniae* productora de CTX-M-15 en infecciones dels tracto urinario de origen comunitario: una epidemiología en evolución. **XIX SEIMC.** Sevilla, 28-30 mayo 2015.

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Emerging extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* causing community-onset urinary tract infections: a case–control–control study

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ABSTRACT

The aim of this study was to determine the epidemiology and risk factors associated with community-onset urinary tract infections (CO-UTIs) due to extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp). A cohort study including all consecutive patients with *K. pneumoniae* CO-UTI identified from January 2010 to December 2014 was conducted. Patients with CO-UTI due to ESBL-Kp were then included as cases in a retrospective case–control–control study; controls were outpatients with CO-UTI caused by non-ESBL-producing *Escherichia coli* and *K. pneumoniae* (non-ESBL-Ec and non-ESBL-Kp, respectively). Each control was matched in a 2:1 ratio according to patient age, sex and year of isolation. Genotyping confirming ESBL was performed by multiplex PCR and sequencing. The prevalence of ESBL-Kp CO-UTIs, calculated among all *K. pneumoniae* CO-UTIs, increased from 2.4% in 2010 to 10.3% in 2014 ($P = 0.01$). Among cases, 63.8% were truly community-acquired, and CTX-M-15 was the predominant β-lactamase enzyme type (79.3%). A total of 83 cases and 319 controls were studied. Being a nursing home resident [odds ratio (OR) = 8.8, 95% confidence interval (CI) 2.6–29.4] and previous cephalosporin use (OR = 4.01, 95% CI 1.8–9.2) were risk factors independently associated with CO-UTI due to ESBL-Kp. In conclusion, the prevalence of CO-UTIs due to ESBL-Kp is increasing. In most cases, ESBL-Kp CO-UTIs are community-acquired and produce CTX-M-15 β-lactamase. Exposure to cephalosporins and being a nursing home resident were risk factors associated with ESBL-Kp CO-UTIs. CTX-M-15-producing *K. pneumoniae* isolates are emerging in the community.

1. Introduction

Antimicrobial-resistant *Klebsiella pneumoniae* has been traditionally recognised as an important nosocomial pathogen affecting mainly severely ill patients. Since the first identification of extended spectrum β-lactamases (ESBLs) able to hydrolyse oxyimino-cephalosporins in the early 1980s, third-generation cephalosporin resistance has been mainly due to ESBL production [1].

ESBLs are plasmid-mediated enzymes that have the ability to hydrolyse narrow and expanded-spectrum cephalosporins and aztreonam, but do not affect cephamycins and carbapenems. These enzymes are inhibited by the so-called 'classical' β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. Frequently, ESBL plasmids also encode other resistance determinants involving fluoroquinolones, trimethoprim/sulfamethoxazole (SXT) and aminoglycosides [2].

The first types of ESBLs described were derivatives of the TEM-1, TEM-2 and SHV-1 enzymes, mainly in *K. pneumoniae* strains associated with nosocomial outbreaks [1]. With the emergence of the CTX-M enzymes in the late 1980s, the epidemiology changed and most ESBL-producing strains were found among community-acquired *Escherichia coli* infections [1,3].

In recent years, a new trend is being observed among *K. pneumoniae*: most nosocomial isolates now produce CTX-M-type

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β-lactamases, specifically CTX-M-15. The epidemiology has somehow changed following emergence of the CTX-M-15-type enzyme among *K. pneumoniae*. Reported nosocomial outbreaks caused by CTX-M-15-producing *K. pneumoniae* are frequently described as widely distributed in general hospital wards rather than limited to specific units, and mortality appears to be lower than that previously described for SHV and TEM outbreaks [4]. Furthermore, CTX-M-15-producing *K. pneumoniae* has also been increasingly recognised in the community [5]. Factors driving this increase are not clear. Although several studies to identify risk factors for community-acquired ESBL-producing Enterobacteriaceae have been published [6,7], most have focused exclusively on CTX-M-producing *E. coli* and little is known about the risk factors associated with CTX-M-15-producing *K. pneumoniae* infections [8].

The aim of the present study was to determine the prevalence, clinical features and risk factors associated with community-onset urinary tract infections (CO-UTIs) due to ESBL-producing *K. pneumoniae* (ESBL-Kp) and to study the molecular epidemiology of ESBL-carrying isolates in the CTX-M era.

2. Materials and methods

2.1. Setting, patients and study design

This study was carried out in the Hospital Universitari Mútua de Terrassa, a 500-bed teaching hospital (mean annual admission of 24,000 patients, serving eight primary care centres) in Barcelona, Spain.

Adult outpatients were identified retrospectively through records of the Microbiology Laboratory, which receives samples from the hospital, a nearby chronic-care hospital, and the primary care centres of an area of *circa* 950,000 inhabitants. Only samples obtained from non-hospitalised patients in the primary care centres were included.

All urinary isolates of *K. pneumoniae* with reduced susceptibility to cephalosporins identified in the Community Department of the Microbiology Laboratory from January 2010 to December 2014 were included. Genotyping confirming ESBL isolates was performed by multiplex PCR. A retrospective case–control–control study was performed to identify risk factors associated with CO-UTIs due to ESBL-Kp. Cases were defined as outpatients with CO-UTI due to ESBL-Kp (one sample per patient) during the study period. To avoid the risk of overestimating the association between antibiotic exposure and resistance, two populations were considered for selection of controls [9]: (i) controls with a CO-UTI syndrome not infected by *K. pneumoniae*; and (ii) controls infected with a susceptible strain of *K. pneumoniae*. The first control group was made up of patients with CO-UTI due to non-ESBL-producing *E. coli*, the commonest aetiology of CO-UTI, considered as the general population (hereafter referred to as non-ESBL-Ec controls). From this population, two control patients per case patient were chosen.

The second base population comprised patients with CO-UTI due to susceptible *K. pneumoniae* (hereafter referred to as non-ESBL-Kp controls); for this population, two control patients per case patient were also chosen. Control patients were randomly selected from both populations and were matched to case patients in a ratio 4:1 (2 non-ESBL-Ec and 2 non-ESBL-Kp controls:1 case) according to age, sex and year of isolation.

Data collected regarding patients included demographics, comorbidities based on the Charlson comorbidity score [10], functional status measured by the Barthel Index [11], healthcare relationship, recurrent UTI, permanent urinary catheter, clinical characteristics and previous antibiotic treatment. Data were collected from the medical charts and electronic medical records of hospital and primary care centres.

The study was approved by the local ethics committee of Hospital Universitari Mútua de Terrassa. Owing to the observational character of the study, written informed consent was not required.

STROBE recommendations were followed to strengthen the reporting of results of this study (Supplementary Table S1).

2.2. Definitions

UTI was defined as the presence of symptoms related to the urinary tract and a positive urine culture ($\geq 10^5$CFU/mL). Asymptomatic patients with positive urine cultures were considered to have asymptomatic bacteriuria (for women, $\geq 10^5$CFU/mL of the same uropathogen in two samples; and for men, $\geq 10^5$CFU/mL in only one sample, $\geq 10^2$CFU/mL if obtained from a permanent urinary catheter). Recurrent UTI was defined as three episodes of UTI in the last 12 months or two episodes of UTI in the last 6 months, including the current episode [12]. Functional dependence was defined as a Barthel Index of <60. Previous antibiotic use was defined as administration of at least a single dose of antibiotic during the previous 3 months; previous exposure to quinolones and cephalosporins was specifically identified and recorded.

Healthcare relationship was defined according to Friedman et al [13]. Hospital-acquired infection was defined as an infection acquired during hospital care that was not present or incubating at admission (infections occurring >48 h after admission were considered nosocomial) or in a patient discharged from hospital in the previous 14 days. Healthcare-associated infection was diagnosed if the patient fulfilled at least one of the following criteria: (i) resided in a nursing home or long-term care facility in the 30 days before the episode; (ii) hospitalised in an acute care hospital for ≥48 h, 90 days before the episode; (iii) attended a hospital or haemodialysis clinic or received intravenous therapy, 30 days before the episode; and/or (iv) wound care, enteral nutrition or healthcare at home, 30 days before the episode. Otherwise, the infection was considered as community-acquired. In the present study, all included cases and controls were non-hospital-acquired infections and were therefore considered as community-onset episodes.

ESBL-Kp strains were considered multiresistant if they were non-susceptible to at least one agent in three or more antimicrobial categories as defined by Magiorakos et al [14].

2.3. Microbiological studies

Samples were plated onto chromID® CPS® agar (bioMérieux, Marcy-l'Étoile, France). Identification and antimicrobial susceptibility testing were performed using MicroScan® (Siemens, Munich, Germany). The double-disk synergy test was used to detect ESBLs. Minimum inhibitory concentration (MIC) breakpoints for resistance were based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [15].

DNA extraction from a pure culture of *K. pneumoniae* isolates was done using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany). Detection of $bla_{\text{CTX-M}}$, bla_{SHV} and bla_{TEM} genes was confirmed by a microarray method (Check-MDR CT103 array; Check-Points, Wageningen, The Netherlands) according to the manufacturer's instructions.

Repetitive element PCR (rep-PCR) was performed using the DiversiLab™ system (bioMérieux) according to the manufacturer's instructions for the molecular epidemiological studies. Results were analysed with DiversiLab™ software using the Pearson correlation coefficient method to determine distance matrices and with unweighted pair-group method with arithmetic mean (UPGMA) pattern matching to determine the clonal relationships and to create dendrograms. A cluster of closely related isolates was defined as

isolates sharing ≥95% similarity, and indistinguishable isolates as sharing ≥97% similarity.

2.4. Statistical analysis

All statistical analyses were performed using STATA Statistical Software Release 14 (StataCorp LP, College Station, TX). The annual prevalence was calculated using as denominator the total number of *K. pneumoniae* isolated from urinary samples from non-hospitalised patients and as numerator the total number of ESBL-Kp isolated in urinary samples (one isolate from each non-hospitalised adult patient included) per year. The annual trend was analysed using a linear adjustment model. Categorical variables are presented using counts and percentages and continuous variables as the mean and standard deviation or the median and interquartile range. Conditional logistic regression was used to compute the crude odds ratio and 95% confidence interval. Multivariate analysis was also performed by conditional logistic regression. Starting with all variables that showed a trend towards an association ($P < 0.2$), the best subset regression procedure was used to identify the most suitable and parsimonious multivariate model, i.e. the one with the lowest Akaike information criterion, which is a well-known parameter of the goodness of fit of the model [16]. Differences were considered statistically significant at a two-sided P-value of <0.05 level. The χ^2 test was used to detect differences between the antibiotic susceptibility pattern of ESBL-Kp cases and non-ESBL-Kp controls.

3. Results

3.1. Retrospective cohort study

The prevalence of CO-UTI due to ESBL-Kp in the study area, calculated among all *K. pneumoniae* CO-UTIs, increased from 2.4% in 2010 (6/253 CO-UTIs caused by ESBL-Kp) to 10.3% in 2014 (30/291) ($P = 0.01$), whilst the prevalence of CO-UTIs due to non-ESBL-Kp, calculated within all CO-UTIs, remained stable (7.5% in 2010 to 8.5% in 2014; $P = 0.08$) (Fig. 1).

3.2. Case–control–control study

During the study period, a total of 83 cases of CO-UTI due to ESBL-Kp and 319 matched controls of CO-UTI (164 due to non-ESBL-Ec and 155 due to non-ESBL-Kp) were analysed; 13 (4.1%) controls had to be excluded (4 because of missing data on clinical charts and 9 due to previous episodes of infections caused by ESBL-producing Enterobacteriaceae).

Table 1 shows the results of the univariate analysis. The interactions tested for both populations were functional dependence; being a nursing home resident; previous hospital admission; Charlson comorbidity score; recurrent UTI; permanent urinary catheter; and previous exposure to antibiotic treatment (cephalosporins, fluoroquinolones or other antibiotics).

Multivariate analysis was done separately for each of the two control groups. With regard to the non-ESBL-Kp population, the following variables were introduced into the multivariate analysis: functional dependence; being a nursing home resident; previous hospital admission; recurrent UTI; and previous exposure to cephalosporins and to fluoroquinolones. The variables independently associated with CO-UTI due to ESBL-Kp are shown in Table 2. With regard to the non-ESBL-Ec population, the following variables were introduced into the multivariate analysis: functional dependence; being a nursing home resident; previous hospital admission; Charlson comorbidity score; recurrent UTI; permanent urinary catheter; and exposure to cephalosporins and to fluoroquinolones. The variables independently associated with CO-UTI due to ESBL-Kp are also shown in Table 2.

The clinical syndrome was unknown in nearly 50% of cases and in two-thirds of both control groups. The most prevalent syndrome was cystitis, followed by asymptomatic bacteriuria, catheter-related UTI, pyelonephritis and prostatitis in all groups.

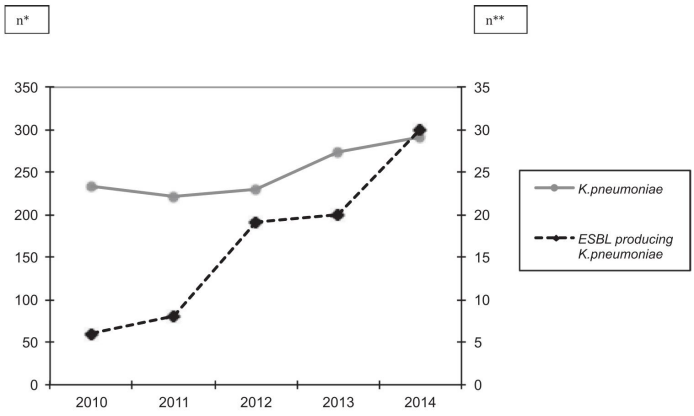

Fig. 1. Annual cases of community-onset urinary tract infection (CO-UTI) due to ESBL-producing *Klebsiella pneumoniae* and non-ESBL-producing *K. pneumoniae*. ESBL, extended-spectrum β-lactamase.

Table 1
Univariate analysis of risk factors for case patients with community-onset urinary tract infection (CO-UTI) due to extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp) compared with control patients with CO-UTI due to non-ESBL-producing *K. pneumoniae* (non-ESBL-Kp controls) and patients with CO-UTI due to non-ESBL-producing *Escherichia coli* (non-ESBL-Ec controls).[a]

Variable	ESBL-Kp cases ($n = 83$)	Non-ESBL-Kp controls ($n = 155$)	OR (95% CI)	*P*-Value	Non-ESBL-Ec controls ($n = 164$)	OR (95% CI)	*P*-Value
Age (years) [median (range)]	78 (14–101)	79 (16–96)			78 (14–99)		
Female sex	57 (68.7)	108 (69.7)			112 (68.3%)		
Functional dependence[b]	24 (30.4)	29 (21.8)	2.15 (1–4.6)	0.050	25 (18)	2.63 (1.2–5.76)	0.016
Healthcare relationship							
Nursing home resident	17 (21.3)	6 (4.1)	5.67 (2.07–15.52)	0.001	8 (5)	12.9 (2.9–56.75)	0.001
Previous hospital admission	13 (15.9)	16 (10.3)	1.93 (0.79–4.73)	0.15	10 (6.1)	2.93 (1.2–7.15)	0.018
True community-acquired	51 (63.8)	127 (85.2)	0.25 (0.11–0.53)	<0.001	143 (88.8)	0.1 (0.035–0.3)	<0.001
Co-morbidities							
Charlson comorbidity score [median (range)]	1 (0–9)	1 (0–11)	1.09 (0.93–1.3)	0.28	1 (0–6)	1.24 (1.04–1.48)	0.016
Recurrent UTI	39 (49.4)	61 (41.2)	1.57 (0.86–2.86)	0.14	54 (38.6)	1.88 (0.99–3.58)	0.054
Permanent urinary catheter	11 (13.9)	14 (9.0)	1.67 (0.68–4.14)	0.26	8 (5.2)	3.41 (1.16–10.02)	0.026
Previous antibiotic treatment							
Cephalosporins	27 (33.3)	16 (11)	4.06 (1.93–8.56)	<0.001	22 (14.3)	2.78 (1.43–5.41)	0.003
Fluoroquinolones	22 (27.2)	20 (13.7)	2.66 (1.24–5.72)	0.012	27 (17.5)	1.91 (0.94–3.87)	0.075
Other antibiotic	14 (17.3)	28 (19.2)	0.95 (0.45–2)	0.9	19 (12.3)	1.4 (0.69–2.83)	0.35

OR, odds ratio; CI, confidence interval.
[a] Data are *n* (%) of patients unless otherwise stated. Percentages are calculated with the number of data available for each variable.
[b] Functional dependence is defined as Barthel Index of <60 [11].

Table 2
Multivariate analysis of risk factors for community-onset urinary tract infection (CO-UTI) due to extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp).

	OR (95% CI)	*P*-Value
Non-ESBL-Kp controls		
Nursing home resident	8.8 (2.63–29.35)	0.000
Previous use of cephalosporins	4.01 (1.75–9.17)	0.001
Previous use of quinolones	3.27 (1.36–7.91)	0.008
Non-ESBL-Ec controls		
Nursing home resident	9.58 (2.07–44.25)	0.004
Previous use of cephalosporins	2.49 (1.16–5.35)	0.019
Previous use of quinolones	2.11 (0.89–4.96)	0.089
Charlson co-morbidity score	1.28 (1.03–1.59)	0.027

OR, odds ratio; CI, confidence interval; non-ESBL-Kp, non-ESBL-producing *Klebsiella pneumoniae*; non-ESBL-Ec, non-ESBL-producing *Escherichia coli*.

The antibiotic susceptibility pattern for ESBL-Kp cases and non-ESBL-Kp controls is shown in Table 3. ESBL-Kp exhibited much higher resistance to all antimicrobials tested; 86.7% (72/83) of the ESBL-Kp were multidrug-resistant.

3.3. Molecular characterisation

Of the 83 ESBL-Kp isolates, 29 (34.9%) were available for molecular characterisation. Seventeen different clonal patterns, designated A–Q, were identified. The most prevalent clonal pattern was F with six isolates, followed by E and L with three and two isolates, respectively. Of the 29 isolates, 23 (79.3%) produced CTX-M-15, 4 (13.8%) produced CTX-M-9 and 2 (6.9%) produced SHV-238S.

Table 3
Antibiotic resistance patterns of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp) cases and non-ESBL-Kp controls.

	Cases ($n = 83$) (%)	Control-Kp ($n = 155$) (%)	*P*-Value
AMC	67 (80.7)	17 (11.0)	<0.001
CTX	80 (96.4)	3 (1.9)	<0.001
CZO	71 (85.5)	3 (1.9)	<0.001
CIP	60 (72.3)	15 (9.7)	<0.001
SXT	60 (72.3)	15 (9.7)	<0.001
FOS	19 (22.9)	30 (19.4)	NS
GEN	35 (42.2)	3 (1.9)	<0.001
NIT	31 (37.3)	22 (14.2)	<0.001
TZP	27 (32.5)	3 (1.9)	<0.001
Multiresistant strains	72 (86.7)	12 (7.7)	<0.001

Note: Figures represent numbers of resistant isolates (% resistance).
AMC, amoxicillin/clavulanic acid; CTX, cefotaxime; CZO, cefazolin; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; FOS, fosfomycin; GEN, gentamicin; NIT, nitrofurantoin; TZP, piperacillin/tazobactam; NS, not significant.

4. Discussion

ESBL-Kp has been traditionally recognised as a common cause of nosocomial infections and hospital outbreaks. Recent data suggest that the presence of ESBL-Kp, and specifically CTX-M-15-producing strains, might be emerging in the community [4]. However, scarce information is available on the epidemiology of these community-acquired infections, and the specific risk factors for CTX-M-producing *K. pneumoniae* have not been well identified.

The present study provides some insights into this evolving epidemiology. Here we report a relevant increase in the prevalence of ESBL-Kp causing CO-UTIs in a large health area, in parallel with an increase in the prevalence of the CTX-M-15-type enzyme among *K. pneumoniae* isolates. These findings are consistent with recent reports confirming the increased presence of ESBL-Kp in the community [17–19].

It is noteworthy that almost two-thirds of the case patients in this study had not been hospitalised during the previous 3 months, had not been in contact with hospital outpatient services and had not been in nursing homes, so their infections can be considered truly community-acquired. Moreover, more than three-quarters of the ESBL-Kp strains were multiresistant. Specifically, most were resistant to fluoroquinolones and SXT.

The high prevalence of CO-UTIs due to CTX-M-15-producing *E. coli* is an established fact. However, for *K. pneumoniae*, until now CTX-M-15 production in the community was anecdotic. The results in the present study represent a new and worrisome scenario in the community setting: the second most frequently isolated agent causing CO-UTI is now also harbouring CTX-M-15, an enzyme until recently associated with *E. coli*. On the other hand, some patients with infections due to *K. pneumoniae* harbouring ESBL seen in the hospital setting are now epidemiologically considered as community-acquired, having occurred among patients with no discernible healthcare-associated risk factors. If these findings are confirmed in other areas, this may change our traditional epidemiological view. In fact, hospital outbreaks originating from a community source of *K. pneumoniae* harbouring ESBL have been already reported [20,21].

To investigate risk factors for resistance among patients with infections due to a specific micro-organism, the control group should be chosen among patients with susceptible bacteria of the same species. However, such a design may overestimate the importance of previous antimicrobial use because patients who had received antimicrobials would probably be under-represented in the control population. This can be avoided by choosing the control patients from among all patients at risk, although some of the identified risk factors might then be non-specifically associated with the risk of developing an infection caused by the susceptible organism [9]. For this reason, a case–control–control design was proposed. A similar design has been used to identify risk factors for bloodstream infections caused by ESBL-producing *E. coli* [22].

Being a nursing home resident and previous cephalosporin use were risk factors in both populations, indicating that they are truly associated with CO-UTI due to ESBL-Kp. Previous use of quinolones was a specific risk factor to CO-UTI due to ESBL-Kp in the non-ESBL-Kp control group, but taking into account that it was not found in the non-ESBL-Ec control group, the association may overestimate the importance of the previous use of quinolones.

The two risk factors identified in this study are similar to those previously reported for community-acquired infections caused by other Enterobacteriaceae, mainly ESBL-producing *E. coli* [3,6,23,24]. However, until now, ESBL-producing *E. coli* and *K. pneumoniae* have been considered to present differences in epidemiological risk profiles, representing differences in transmission dynamics [8].

These findings may suggest that acquisition of the CTX-M-15-type enzyme by *K. pneumoniae* in community isolates may resemble the epidemiological phenomenon described years ago with *E. coli*, suggesting that the interface between hospitals and the community is becoming blurred. There are many examples of the presence of *K. pneumoniae* in the community: there are reports of faecal carriage of ESBL-Kp in outpatients and healthy individuals in many geographical areas [5,25], and tap water and treated waste water as well as food have been identified as possible sources of *K. pneumoniae* harbouring CTX-M-15 [26,27].

CTX-M-15 was the most common type of ESBL identified among cases in the present study. CTX-M enzymes have epidemically expanded among Enterobacteriaceae and are now the dominant ESBL type among clinical isolates, mainly among *E. coli* [28]. An important factor in their global dominance is the wide dissemination of bacterial clones producing CTX-M-type ESBLs. In fact, the clonal dissemination of CTX-M-15-producing *E. coli* belonging to phylogenetic group B2 and sequence type 131 has been identified as the most prevalent in many countries [29]. Remarkably, no clonal dissemination was detected in the cohort in the current study, in contrast to the community-acquired ESBL *E. coli* epidemics described previously. Probably, the rapid emergence of CTX-M-15 as one of the most important epidemic ESBLs in *E. coli* has generated a large genetic reservoir from which other species, such as *K. pneumoniae*, could easily acquire this resistance gene [30]. However, with *K. pneumoniae* it appears that resistance is not restricted to a few genetic backgrounds and that it is a phenomenon of multiple emergence rather than the spread of a few clones [31].

This study has some limitations. Data were collected retrospectively from medical charts and electronic medical records in hospital and primary care settings, therefore it was difficult to delineate with accuracy the clinical syndrome owing to missing information. Patients from the emergency department were not included, which may have contributed to an under-representation of community-acquired cases. Matching was performed according to age and sex in order to avoid confounding with co-morbidities and the high prevalence of lower UTI in women, respectively. In consequence, these two variables have not been investigated as risk factors. Healthcare relationship was defined according to the criteria of Friedman et al [13]. Although the criteria were not modified in accordance with the epidemiology of the UTI, relevant variables (recurrent UTI, permanent urinary catheter) were collected. Despite our best efforts, only one-third of samples were available for enzyme characterisation, and plasmid analysis was not performed. Finally, this study was conducted in an urban teaching institution with a very large outpatient population and the results cannot be extrapolated to other settings.

Despite the above limitations, this study has shown a remarkable increase in the prevalence of ESBL-Kp in the community and that previous exposure to cephalosporins as well as being a nursing home resident are clearly linked to the isolation of ESBL-Kp causing UTI in the outpatient population. CTX-M-15 enzymes appear to have replaced older types of ESBLs and to have become the predominant enzymes among several different *K. pneumoniae* clones.

The results of this study may have some practical consequences. First, interventions directed at reducing the use of cephalosporins for the treatment of CO-UTIs whenever possible should be pursued. Second, when treatment protocols are designed, it might be prudent to consider empirical therapy with agents active against ESBL-producing organisms in high risk-patients with severe community-acquired urinary tract sepsis. Third, hospital infection control measures should contemplate the evolving epidemiology of CTX-M-15-producing *K. pneumoniae*.

5. Conclusions

The prevalence of CO-UTI due to ESBL-Kp is increasing. CTX-M-15 enzymes appear to have replaced older types of ESBLs in *K. pneumoniae* and to have become the predominant enzymes among several different clones. Remarkably, almost two-thirds of cases were truly community-acquired. The identified risk factors for CO-UTI due to ESBL-Kp were exposure to cephalosporins and being a nursing home resident.

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Ethical approval: The study was approved by the local ethics committee of Hospital Universitari Mútua de Terrassa (Barcelona, Spain).

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2017.03.009.

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Antimicrobial Resistance and Infection Control

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High clonal diversity of ESBL-producing *Klebsiella pneumoniae* isolates from clinical samples in a non-outbreak situation. A cohort study

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Abstract

Background: *Klebsiella pneumoniae* has been responsible for a large number of clonal hospital outbreaks. However, some epidemiological changes have been observed since the emergence of CTX-M enzymes in *K. pneumoniae.*

Aim: To analyse the transmission dynamics of Extended Spectrum β-Lactamase-producing *Klebsiella pneumoniae* (ESBL-Kp) in an acute care hospital.

Methods: In 2015 a prospective cohort study was conducted. All new consecutive adult patients with ESBL-Kp isolates in all clinical samples were included. Patients with a previous known infection/colonization by ESBL-Kp and patients in high risk areas (e.g., intensive care units) were excluded. Cross-transmission was defined as the carriage of a clonally-related ESBL-Kp between newly diagnosed patients who shared the same ward for ≥48 h with another case, within a maximum time window of 4 weeks. ESBL-production was confirmed using the double-disk diffusion method and PCR. Clonal relationships were investigated by rep-PCR and multilocus sequence typing (MLST).

Results: Sixty *ESBL-Kp* isolates from 60 patients were included and analysed. Infections and colonizations were classified as hospital-acquired (52%), healthcare-related (40%) or community-acquired (8%).
High genetic diversity was detected. When epidemiological clinical data were combined with the rep-PCR, the patterns identified did not show any cases of cross-transmission. ESBL-Kp were detected in 12.5% of environmental samples. No clonal relationship could be established between environmental reservoirs and patients. The genetic mechanism detected in all strains was associated with *bla* $_{CTX-M}$ genes, and 97% were CTX-M-15.

Conclusions: The dynamics of ESBL-*K. pneumoniae* isolated in our setting could not be explained by clonal transmission from an index patient. A polyclonal spread of ESBL-Kp was identified.

Keywords: ESBL, *Klebsiella pneumoniae*, Clonal diversity

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Introduction

The epidemiology of healthcare-related infections has been characterized in recent decades by the emergence of Gram-negative multidrug-resistant organisms [1]. This increase in resistance appears to be due largely to the production of extended-spectrum β-lactamases (ESBLs) among all Enterobacterales. ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) is one of the most frequently identified multiresistant pathogens.

K. pneumoniae has been responsible for a large number of hospital outbreaks. In the 1990s, these outbreaks were clonal epidemics affecting mainly intensive care patients, and were due to SHV [2] and TEM enzyme types [3]. The first reports of CTX-M *K. pneumoniae* outbreaks were published in the 2000s [4]. Conversely, these CTX-M outbreaks were widespread in general hospital wards and their mortality rates are lower than those previously associated with SHV and TEM outbreaks.

Since the emergence of CTX-M β-lactamases, several clones harboring CTX-M-15 enzymes, often associated with other ESBL types, have been identified [5]. In the case of *K. pneumoniae* it seems that resistance is not restricted to a few genetic backgrounds, and that it is a phenomenon of multiple emergence rather than one involving the spread of a few clones [6]. In fact, high clonal diversity has been reported in non-outbreak [7] and outbreak [8] situations.

In the last 5 years, we have detected an increase in the incidence of hospital-acquired ESBL-Kp infections in our area (from 0.06 in 2011 to 0.35/1.000 stays in 2015) alongside a rise in the prevalence of community-acquired urinary tract infection due to ESBL-Kp (from 2.4% in 2010 to 10.3% in 2014). Most of these clinical isolates harbored CTX M-15 enzymes [9].

With the increase in ESBL-Kp among community-acquired cases [10, 11] and in the hospital setting [4, 12–14] there is a clear need to understand the dynamics of transmission of this relevant pathogen. It is crucial to determine whether the isolation of ESBL-Kp 48 h after hospital admission is actually caused by hospital cross-transmission, and also the extent to which it is preventable and merits infection control interventions.

In this scenario, the aim of the present study was to investigate the dynamics of transmission of ESBL-producing *Klebsiella pneumoniae* by assessing both the clinical epidemiological data and the clonal relatedness of ESBL-Kp among inpatients at a single academic acute care hospital.

Material and methods

Setting and study design

In 2015 a prospective cohort study was conducted at Hospital Universitari Mútua Terrassa, Barcelona, a 400-bed acute care hospital with an annual mean number of 97,524 hospital stays for a population of 350,000 inhabitants. Patients are hosted in single or double rooms. Bathrooms are shared in double rooms.

Inclusion criteria

All new consecutive adult patients with ESBL-producing *Klebsiella pneumoniae* isolates from any specimens obtained by routine clinical practice were included in the study (one sample/patient). Patients with previous known infection/colonization by ESBL-Kp were excluded, as were adult patients admitted to intensive care units (ICU).

Clinical epidemiological data collection and infection control standards

Definitions:

- "Index patient" was defined as an inpatient with a newly recognized clinical sample yielding ESBL-Kp.
- "Contact patient" was defined as a person who shared the same room for ≥ 24 h with an index patient without initiation of contact precautions.
- "Cross-transmission" was defined as the carriage of a clonally-related ESBL-Kp among newly diagnosed patients sharing the same room or ward for ≥48 h with another index case, within a maximum time window of 4 weeks.
- "Healthcare relation" was defined according to *Friedman* et al. [15] "Hospital-acquired infection" was defined as an infection acquired during hospital care that was not present or incubating at admission (infections occurring 48 h after admission were considered) or in a patient discharged from hospital in the previous 30 days. "Healthcare-related infection" was diagnosed if the patient fulfilled at least one of the following criteria: (i) having resided in a nursing home or long-term care facility in the 30 days before the episode; (ii) hospitalized in an acute care hospital for ≥48 h in the 90 days before the episode; (iii) having attended a hospital or hemodialysis clinic or received intravenous therapy in the 30 days before the episode; and/or (iv) having received intravenous therapy, wound care, enteral nutrition or healthcare at home in the 30 days before the episode. Otherwise, the infection was classified/considered as community-acquired.

All index patients were placed on contact precautions. Index patients were screened at the time of first detection in order to determine colonization. Screening samples included a rectal swab and urine sample in patients with a Foley catheter. According to ESCMID guidelines [16], three or more repeatedly negative screening cultures over the course of one or 2 weeks in a patient who had not received antimicrobial therapy for several weeks

were needed to consider that a patient was decolonized and, therefore, that contact precaution measures could be suspended. No active decolonization policies were conducted. Contact precautions included a single-patient room and the use of gloves and gowns by healthcare workers.

Basic infection control standards included proper hand hygiene (as indicated in the WHO guidelines) [17]. In 2015, compliance with a hospital-wide project promoting hand hygiene was 64%. During the study period a stringent environmental cleaning process including twice-daily hospital cleaning with detergents, as well as enhanced terminal cleaning of rooms of targeted patients on contact precautions, was also conducted.

Infection control staff routinely visited all inpatients with colonization or infection due to ESBL-producing *Klebsiella pneumoniae*. Data on demographics, type of sample, healthcare-relatedness, time from admission until ESBL-Kp identification and movements around the hospital (including detailed information regarding rooms and wards occupied during the hospital stay) were prospectively collected as part of the standard epidemiological clinical work-up conducted by the infection control team. Rectal swab screening for contact patients was also performed as well. All patients with known ESBL carriage were screened whenever they were re-admitted to the hospital as part of standard infection control practices. No other active surveillance was applied.

Environmental samples

A surveys of environmental colonization of ESBL-Kp were performed in some of the rooms occupied by ESBL-Kp colonized or infected patients during the year under study. Four samples were obtained in each surveyed room (tap of the sink, surface around the sink, bedpan and bedpan washer tap) [18].

Samples were obtained for culture by rubbing gauzes moistened with thioglycolate broth repeatedly over designated sites in the immediate vicinity of the patient environment and they were stored in screw-cap sterile containers with 10 mL of thioglycolate broth. The containers were incubated for 24 h at 37 °C and then inoculated onto ChromID ESBL (bioMérieux) [4, 19].

Microbiological methods

Bacterial identification and susceptibility testing was performed using Vitek2 System (BbioMérieux). EUCAST breakpoints were used for interpretation of the results. ESBL-production was confirmed using the double-disk diffusion-method. ESBL characterization was performed by commercial PCR (Check-MDR CT103XL, Hain).

The genetic relationship between all 60 ESBL-Kp isolates was determined by automated repetitive-sequence-based PCR using the Diversilab system (bioMérieux), following the manufacturer's recommendations. Rep-PCR fingerprinting profiles were compared and analyzed by Diversilab (version 3.6) software using Pearson correlation coefficient pairwise pattern matching and the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. The cutoff value for similarity in order to establish strain identity was 95%.

Multilocus sequence typing (MLST) was performed using seven conserved housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB* and *tonB*) [20]. The protocol of the MLST procedure, including allelic type and sequence type (ST) assignment methods, is available from the MLST databases of the Pasteur Institute (Paris, France) http://bigsdb.pasteur.fr/klebsiella/klebsiella.html. The phylogenetic relationships between the different ST found in the study were established by Phyloviz (https://online.phyloviz.net/index) using the goeBURST algorithm.

Results

During 2015, 60 consecutive index cases were identified. Demographic and clinical data of patients and isolates are shown in Table 1. In order of frequency, the origins of the clinical samples were urine (47, 78%), surgical wounds (six, 10%), blood (six, 10%) and respiratory samples (one, 2%). Thirty-two isolates were obtained from patients admitted to the hospital emergency department, 16 in surgical wards and 12 in medical wards.

New index cases were detected with a median frequency of 2.5 (range 0–6) patients per month and there were no outbreaks in any specific hospital area (Fig. 1). Counting all *K. pneumoniae* isolates, the proportion of samples with ESBL-producing enzymes in our hospital in 2015 was 26.10%, compared with 27.52% in Catalonia as a whole [21].

Among the clinical samples, 47 (78.3%) were interpreted as infections and 13 (21.7%) as colonizations.

Hospital-acquired infection/colonization was demonstrated in 31 index cases (52%), healthcare-related infection/colonization in 24 (40%) patients, and community-acquired infection/colonization in five (8%). Among healthcare-related samples, 11 (17%) were from nursing home residents. At two particular nursing homes two cases were identified, but neither a temporal nor a genetic relationship could be established between the isolates in either setting.

High genetic diversity was detected. The isolates were classified into 36 different patterns (rep-PCR, Diversilab); only 4/36 patterns included three or more isolates. Sixteen sequence types (ST) were identified. The most prevalent STs encountered were ST170 (23%), ST405 (21%) and ST392 (16%). Altogether, these STs represented 60% of the isolates. A summary of the

Table 1 Demographics and clinical characteristics of patients and isolates

Patients characteristics	Community-acquired	Healthcare-related	Hospital-acquired infection	Total
Number of isolates and patients	5	24	31	60
Gender, male (N)	1	12	20	33
Sample sites (N)				
- Urine	5	21	21	47
- Wounds	0	1	5	6
- Blood	0	2	4	6
- Respiratory	0	0	1	1
Units (N)				
- Surgery wards	1	2	13	16
- Medical wards			12	12
- Emergency department	4	22	6	32

characterization of the 60 isolates is shown in Fig. 2, and the phylogenetic relationships between the different STs are shown in Fig. 3. See Additional files 1, 2 and 3, available as Supplementary information, with the characteristics of each isolate according the site of acquisition.

Thirty-one strains with 19 different rep-PCR patterns and eight different STs were identified among hospital-acquired infection samples. The most frequent were ST170 (26%), ST405 (26%) and ST392 (23%).

Among healthcare-related cases, 24 strains were collected with 22 different patterns identified by rep-PCR and 11 STs. In this case the most frequent were ST170 (25%), ST405 (21%) and ST392 (13%).

Five community-acquired samples were included which showed five different patterns by rep-PCR and MLST. Two community-acquired sequence types (ST70 and ST307) were also found in two healthcare-related strains and in three hospital-acquired infection strains.

No cases of cross-transmission were found when epidemiological clinical data were combined with the rep-PCR patterns identified (Fig. 1).

The genetic mechanism detected in all ESBL-Kp isolates was associated with the presence of *bla* $_{\text{CTX-M}}$ genes. Most of the CTX-M detected belonged to group 1: CTX-M-15 (58 strains) and CTX-M-32 (one strain). One strain belonged to group 9. No carbapenemase producers were detected. All ESBL enzymes in hospital-acquired and community-acquired samples were CTX-M-15, as were 22 out of 24 identified in healthcare-related strains (the exceptions being one CTX-M-9 and one CTX-M-32).

Antibiotic susceptibility testing showed that all isolates were resistant to cefotaxime and ceftazidime. The antimicrobial resistance pattern is summarized in Table 2. The phenotypical characterization showed differences between isolates with the same sequence type.

Environmental samples isolated

ESBL-Kp were detected in 4/32 (12.5%) environmental samples from three rooms.

One room had two positive samples (surface around the sink and bedpan washer tap) with an identical rep-PCR pattern, though it was not identified with a particular patient.

The other positive samples were isolated in two different rooms. The first one was on the surface around the

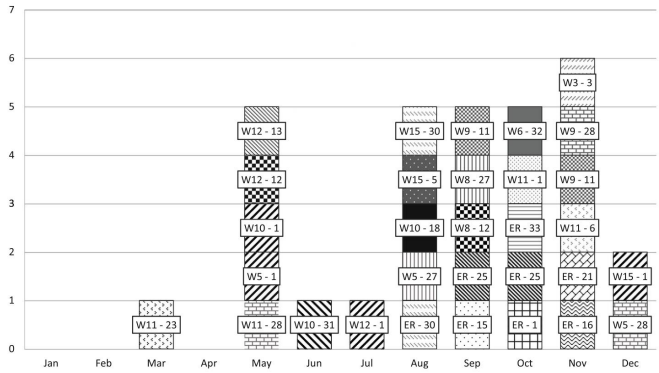

Fig. 1 Rep-PCR pattern of ESBL-*K. pneumoniae* isolates of hospital-acquired infection origin per month and ward. Each square represents the number of isolates; every rep-PCR pattern is represented by a different drawing and number (1–33). All the isolates are labelled according to the ward (W: 5 to 15) or ER (Emergency room) and rep-PCR pattern (number)

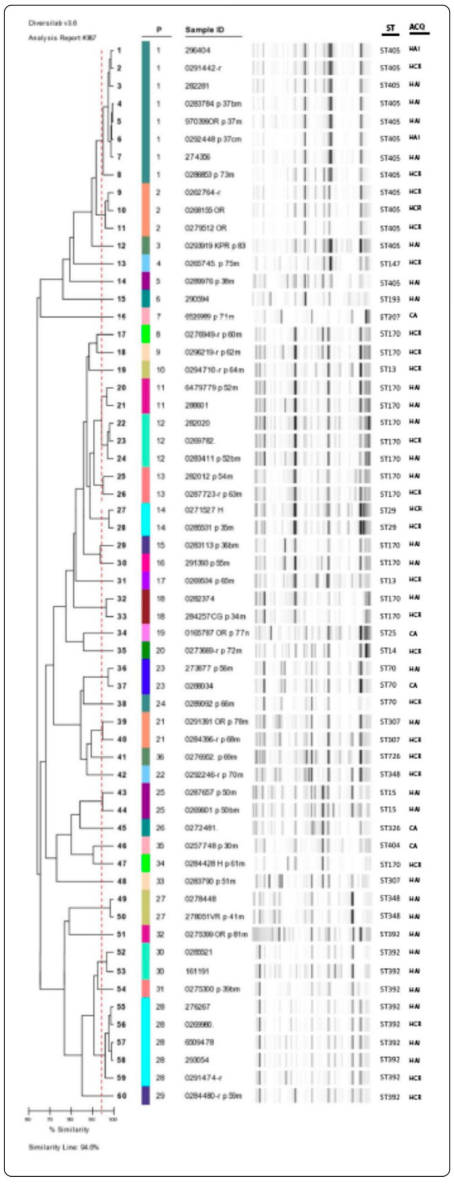

Fig. 2 Cluster analysis and virtual gel image from DiversiLab generated fingerprints of the 60 *K. pneumoniae* strains, including corresponding ST results from MLST. ACQ: Site of acquisition/HAI:hospital-acquired infection/HCR:healthcare-related/CO:community-acquired

sink with the same rep-PCR pattern as the strain isolated in the previous occupant of the room; the second was cultured from a sink tap and presented a rep-PCR pattern different from the one identified in a patient who had previously occupied the room.

Discussion

In a non-outbreak setting, no cases of cross-transmission of ESBL-producing *Klebsiella pneumoniae* could be demonstrated in general wards (non-ICU) in our hospital during the year under study. A high genetic diversity was confirmed by both rep-PCR and MLST.

Traditionally, ESBL-*Kp* cross-transmission via the hands of healthcare workers [2] and the lower gastrointestinal tract of colonized patients [22] has been documented as the main reservoir of these microorganisms during hospital outbreaks [5, 23]. However, in our setting, we could not demonstrate either a clonal or a clinical epidemiological relatedness between consecutive non-duplicate ESBL-*Kp* strains isolated during 2015. Therefore, this traditional dynamics cannot explain our epidemiology. A low rate of hospital-acquired infection transmitted by highly drug-resistant Gram-negative bacteria was also found in a large multicenter trial involving 18 Dutch hospitals, and in a single-center Swiss study of ESBL-producing Enterobacterales [24, 25].

Interestingly, a recent study showed that only half of the cases of healthcare-acquired infection or colonization due to multi-drug resistant organisms (MDRO) according to CDC definitions are truly hospital-acquired [26]. Similarly, some reports suggest that some patients with infections caused by ESBL-producing *K. pneumoniae* isolates seen at hospitals should be epidemiologically defined as community-associated [9, 11]. Therefore, in these scenarios, ESBL-producing *K. pneumoniae* is more likely to have been imported into the hospital than to have originated there. In fact, hospital outbreaks originating from a community source of ESBL-producing *K. pneumoniae* have already been described [4].

However a word of caution is in order before concluding that there is no transmission. Carriership is generally asymptomatic and universal screening is not conducted at our hospital. As a result, intermediate patients may be missed and no epidemiological link can be made. In addition, a seasonal variation was identified in our study. Nevertheless, it was recently shown that in a non-outbreak setting, importation of ESBL-producers into hospitals seems to be at least as frequent as transmission

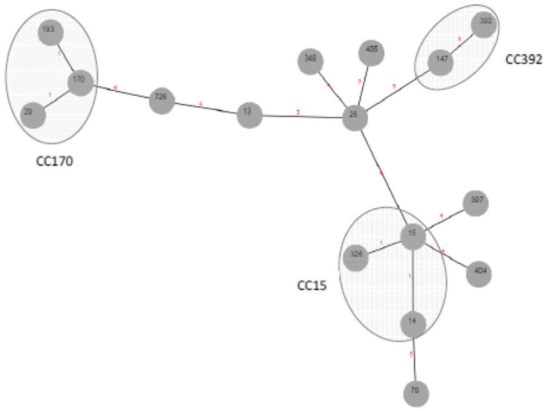

Fig. 3 Phylogenetic relationships between the STs detected in our study. ST29 and ST193 are single-locus variant of ST170 as well as, ST 326 and ST14 are single-locus variant of ST15. Numbers circled indicate the ST and the number on the branch indicates the number of different alleles between STs. CC: clonal complex

events during the hospital stay [27]. The total lack of clonal relatedness between index cases and contact patients makes cross-transmission highly improbable in our setting. It should also be stressed that for financial reasons universal screening for all MDRO is not carried out at most acute care hospitals.

Hospital environmental contamination has been reported as the source of several ESBL-Kp outbreaks [4, 28, 29]. In our study, no clonal relationship with environmental samples could be established. However, it is conceivable that more extensive environmental screening would have identified a reservoir possibly missed by the present design.

Only cross-transmitted MDRO are preventable and are reasonable targets for an infection control program. Non-preventable events may be related to selective antibiotic pressures that trigger the emergence of ESBL-producing *K.pneumoniae* colonizing the gastrointestinal tract after admission. In this scenario, infection control measures must be coordinated with antimicrobial stewardship programs to stop the endemic evolution of ESBL-producing *K. pneumoniae*.

Table 2 Antimicrobial resistance of *K. pneumoniae* ESBL-producing isolates

Antibiotic	Community- acquired (*N* = 5)	Healthcare- related *N* = 24	Hospital- acquired infection (*N* = 31)	Total
Amoxicillin-clavulanic acid	3/5	21/24	31/31	55/60
Amikacin	0/5	2/24	1/27	3/57
Cefepime	5/5	24/24	31/31	60/60
Cefuroxime	5/5	24/24	31/31	60/60
Cefotaxime	5/5	24/24	31/31	60/60
Ceftazidime	5/5	24/24	31/31	60/60
Ciprofloxacin	3/5	21/24	31/31	55/60
Ertapenem	0/5	0/24	0/31	0 / 60
Gentamicin	1/5	13/24	14/31	28 /60
Imipenem	0/5	0/24	0 /31	0 /60
Piperacillin/tazobactam	2/5	12/24	20/31	34/60
Trimethoprim/sulfamethoxazole	3/5	21/24	29/31	53/60

Regarding the antibiotic resistance phenotype, isolates were mostly classified as CTX-M 15 producers. These results are in agreement with those previously reported in other countries and thus corroborate the wide distribution of this enzyme [12, 30–33].

The STs identified in our setting belong to previously described international clones associated with multidrug-resistant *K. pneumoniae* isolates. ST170 was the most frequent sequence type identified in our cohort. To our knowledge, this is the first report of ST170 in human strains. Moreover, ST170 was only detected in hospital strains without any epidemiological relationship, suggesting the possibility of an endemic situation. The other two frequently identified STs in our cohort (ST405 and ST392) have been described elsewhere in Europe and in South America in strains of human origin.

Machuca et al. [34] published the first report of a *K. pneumoniae* ST405 without harbouring a carbapenemase, the type we found in our isolates. Previously, ST405 has been described as a clone capable of disseminating different quinolone and beta-lactam resistance determinants (including ESBL and carbapenemase): in Spain and France among OXA-48 and CTX-M15- producing isolates, and in Yemen among NDM and CTX-M-15 producers. ST392 has been reported in *K. pneumoniae* in CTX-M-15 associated with carbapenemase: in KPC in China, and in OXA-48 in Europe. This is the first time that ST392 has been described in *K. pneumoniae* carrying only CTX-M.

The phenotypic method, consisting in the identification of species type and resistance towards several selected antibiotics, was unable to detect ST or rep-PCR groups. This suggests that the phenotypic method is not suitable for infection control procedures and that molecular identification is crucial for the definition of cross-transmission. Similar results were published by Souverein et al. [8].

This study has some limitations. First, the single-center study design may limit the generalizability to other settings and we cannot rule out the possibility that the lack of transmission at our institution may be attributable to the high level of infection control and to the low number of beds per room.

Second, no plasmid typing was performed. The criteria for cross-transmission in the present study did not address the possibility of horizontal transmission of common plasmids between different Enterobacterales species [6, 35].

Third, the gold standard assay for molecular typing is pulsed-field gel electrophoresis (PFGE), due to its high discriminatory power. The discriminatory power of rep-PCR is generally similar to that of PFGE. PCR methods are preferable in the study of small, time-limited outbreaks, while in complex outbreaks of longer duration, in which clonal evolution and dynamics are studied, PFGE should be used. Molecular typing methods based on DNA sequencing such as MLST are applicable in global epidemiological studies [36]. The initial assessment in our study was made using the rep-PCR, and the MLST method confirmed the diversity in our population.

Fourth, the lack of systematic active surveillance of all inpatients admitted or discharged from hospital may have meant that some transmission events were missed. However, systematic surveillance of all contact patients did not demonstrate cross-transmission in this high-risk situation. Fifth, the detection method, i.e., screening for colonization merely by collecting rectal swabs without any enrichment to increase the detection sensitivity, may have missed some ESBL-KP strains in contact patients. Finally, since we only studied ESBL-Kp in a non-outbreak scenario, it may not be possible to extrapolate our results to other Enterobacterales or to other epidemic settings.

In conclusion, in this epidemiological study of a non-outbreak setting, we identified a polyclonal spread of ESBL-Kp with high genetic diversity. Neither clonal cross-transmission nor environmental reservoirs could be demonstrated. Our data suggest that the probable importation of ESBL-Kp into the hospital may explain the dynamics of hospital-acquired cases in our setting. These findings question the validity of the contact precaution measures applied to control ESBL-Kp epidemics. More studies are now required to explore this matter further.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13756-019-0661-9.

Additional file 1. Characteristics community-acquired isolates.
Additional file 2. Characteristics healthcare-related isolates.
Additional file 3. Characteristics hospital-acquired isolates.

Abbreviations

CDC: Centers for Disease Control and Prevention; ESBL: Extended-spectrum β-lactamases; ESBL-Kp: Extended-spectrum β-lactamases-*Klebsiella pneumoniae*; ESCMID: European Society for Clinical Microbiology and Infectious Diseases; EUCAST: European Committee on antimicrobial susceptibility testing; ICU: Intensive care unit; MDRO: Multi-drug resistant organisms; MLST: Multilocus sequence typing; PCR: Polymerase chain reaction; ST: Sequence type; WHO: World Health Organization

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Authors' contributions

MX and EC designed the study. MX, EJ, EP, JP were responsible for performing all laboratory tests. MX, MR, NF were responsible for data collection. MX, EJ, EP, LB-P, EC analysed the data. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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