Universidade Federal de Pernambuco
Centro de Ciências Biológicas
Curso de Mestrado em Bioquímica

DETECÇÃO DE BETA-LACTAMASES DE ESPECTRO ESTENDIDO EM ISOLADOS CLÍNICOS BACTERIANOS

EDUARDO ANTONIO MACIEL DE SOUSA SILVA

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Dissertação apresentada ao curso de Mestrado em Bioquímica do Centro de Ciências Biológicas, da Universidade Federal de Pernambuco, como parte dos requisitos para obtenção do grau de Mestre em Bioquímica.

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Ata da defesa de dissertação do Mestrando **Eduardo Antonio Maciel de Sousa Silva,** realizada em 22 de fevereiro de 2006, como requisito final para obtenção do título de Mestre em Bioquímica

Às 09:30 horas, do dia 22 de fevereiro de 2006, foi aberto, no Auditório Prof. Marcionilo Barros Lins, Depto. de Bioquímica/CCB/UFPE, o ato de defesa de dissertação do mestrando **Eduardo Antonio Maciel de Sousa Silva**, aluno do Curso de Mestrado em Bioquímica/CCB/UFPE. Iniciando os trabalhos a Profa. Dra. Patrícia Maria Guedes Paiva, Vice-Coordenadora do Curso supra citado, em substituição por motivo de doença, a Profa. Dra. Vera Lúcia de Menezes Lima, fez a apresentação do aluno, de sua orientadora Profa. Dra. Vera Lúcia de Menezes Lima, de sua Co-orientadora, Profa. Dra. Márcia Maria Camargo de Morais e da Banca Examinadora composta pelos professores doutores: Luana Cassandra Breitenbach Barroso Coelho, na qualidade de Presidente, do Depto. de Bioquímica/CCB/UFPE, Márcia Maria Camargo de Morais, do Depto. de Patologia/ICB/UPE e Ana Catarina de Souza Lopes, do Depto. de Medicina Tropical/UFPE. Após as apresentações, a Profa. Dra. Patrícia Maria Guedes Paiva passou a palavra a Presidente da Banca que convidou o aluno para a apresentação de sua dissertação intitulada: **"Detecção de Beta-Lactamases de Espectro Estendido em Isolados Clínicos Bacterianos",** e informou que de acordo com o Regimento Interno do Curso, o candidato dispõe de até 50 (cinqüenta) minutos para apresentação do trabalho e o tempo de argüição para cada examinador, juntamente com o tempo gasto pelo aluno para responder às perguntas será de 30 (trinta) minutos. O aluno procedeu a explanação e comentários acerca do tema em 25 (vinte e cinco) minutos. Após a apresentação do mestrando, a Sra. Presidente concedeu um intervalo de 15 minutos. Reiniciando os trabalhos, a Sra. Presidente convidou a Banca Examinadora para ocupar seus lugares e passou a palavra ao primeiro examinador, Profa. Dra. Márcia Maria Camargo de Morais, que agradeceu o convite, fez alguns comentários e deu algumas sugestões, iniciando sua argüição. Ao final, a referida professora deu-se por satisfeita. Em seguida, a Sra. Presidente passou a palavra para a Profa. Dra. Ana Catarina de Souza Lopes, que agradeceu o convite, fez alguns comentários e sugestões, e iniciou sua argüição. Ao final, a referida professora deu-se por satisfeita. Em seguida, a Sra. Presidente usou da palavra para tecer alguns comentários, agradecer à Banca Examinadora e parabenizar o candidato. Finalmente, a sessão foi suspensa para proceder ao julgamento pela Banca Examinadora, a qual se reuniu na Secretaria do Curso. Após alguns comentários, a Banca decidiu, por unanimidade, conceder a menção **"Aprovado com Distinção"**. Nada mais havendo a tratar, lavrei a presente ata que vai assinada por mim, Secretário, e demais membros da Banca Examinadora. Recife, 22 de fevereiro de 2006.

DEDICATÓRIA

À Deus nosso Pai Maior de infinita bondade e sabedoria que através da sua lei manifesta sua justiça e amor para o crescimento de nossas vidas dando-nos a oportunidade de crescer em ciência e em moral visando incansavelmente o progresso da humanidade.

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SUMÁRIO

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LISTA DE ABRAVIATURAS

bla $_{CTX}$ – beta lactamase CTX

bla $_{SHV}$ – beta lactamase SHV

bla $_{TEM}$ – beta lactamase TEM

ESBL – beta-lactamase de espectro estendido

NCCLS – National Committee Clinical Laboratory Standards

PCR – Reação em cadeia da Polimerase

Da – Dalton

UTI – urinary tract infection

ITU – infecções do trato urinário

RESUMO

As beta-lactamases de espectro estendido estão em expansão mundial, conferindo em isolados clínicos importantes fenótipos de multi-resistencia em bactérias que abrigam estas enzimas. Pacientes com infecção urinária (ITU) são principalmente infetados pelas *E. coli* e *K. pneumoniae*, que são as principais produtoras das ESBLs (beta-lactamases de espectro estendido). Neste trabalho, nós analisamos a presença de ESBL produzidas pela *E. coli* e *K. pneumoniae* em isolados de ITU de origem nosocomial e em comunidade. Padrões de suscetibilidade e a presença de genes de ESBL para SHV, TEM e CTX foram estudados em amostras de ESBL positivas. Os resultados mostraram que as cepas produtoras de ESBL estavam presentes em 25% das amostras de ITU nosocomiais e 9.3% nas de comunidade. As ESBLs esteve presente em 12.2 e 18.2% dos isolados de *E. coli* e de K.*pneumoniae*, respectivamente. Foram descobertos genes de ESBL em isolados de origem nosocomial e em comunidade. Todos os isolados que carregam os genes de ESBL apresentaram fenótipos de multi-resistência que alertam à necessidade de vigilância contínua nas ITU.

Palavras-chave: ESBL, infecções do trato urinário, *E.coli, K. pneumoniae*

ABSTRACT

Extended spectrum beta-lactamases are already wideworld spreaded, conferring clinical important multi-resistance phenotypes to bacteria harboring those enzymes. Patients with UTI are mainly infected with *E. coli* and *K. pneumoniae*, the most frequent ESBL producing strains. In this work, we analysed the presence of ESBL producing strains of *E. coli* and *K. pneumoniae* isolated from nosocomial and acquired-community UTI. Susceptibility patterns and the presence of ESBL genes SHV, TEM and CTX were studied in ESBL positive strains. The results showed that ESBL producing strains were present in 25% of nosocomial and 9.3% of acquired-community UTIs. ESBLs were produced by 12.2 and 18.2 % of *E. coli* and *K. pneumoniae* isolates, respectively. ESBL genes were detected in both nosocomial and community-acquired isolates. All the isolates carrying multiple ESBL genes showed multi-drug resistance phenotypes, which alerts to the necessity of continuous surveillance programs in UTIs

Key words: ESBL, urinary tract infections, *E.coli, K. pneumoniae*

1.0 INTRODUÇÃO

1.1 BETA-LACTAMASES

As beta lactamases têm sido designadas pelo Comitê de Nomenclatura Internacional em Bioquímica (Nomenclature Committee of the International Union of Biochemistry) como enzimas que hidrolisam amidas, amidinas ou outras pontes C-N, separando as amidas cíclicas do anel beta-lactâmico (Fig. 01). Algumas beta-lactamases utilizam zinco para romper o anel beta-lactâmico; entretanto, a maioria age via éster de serina (GHUYSEN, 1993).

Primeiramente a enzima associa-se de forma não covalente ao anel beta-lactâmico do antimicrobiano, e então o radical hidroxila livre do resíduo de serina presente no sítio ativo da enzima, ataca o anel beta-lactâmico, formando uma ligação covalente acil-éster. A hidrólise do éster formado libera a enzima ativa e os antimicrobianos hidrolisados e inativos (LIVERMORE, 1995).

1.2 HISTÓRICO DAS BETA-LACTAMASES

A emergência da resistência aos antibióticos beta-lactâmicos iniciou-se logo após o desenvolvimento do primeiro antibiótico beta-lactâmico, a Penicilina, extraída do fungo *Penicillium* sp. (Fig. 02) A primeira produção de Beta-lactamase foi detectada em *Escherichia coli* (Fig. 03) antes mesmo da redução do uso medicinal da Penicilina. O uso da Penicilina favoreceu uma grande emergência de resistência em *Staphylococcus aureus* através de plasmídios contendo genes da Penicilinase. Esta beta-Lactamase rapidamente se disseminou e é muito encontrada atualmente em isolamentos clínicos de *Staphylococcus aureus* e em outras espécies do gênero *Staphylococcus* (ABRAHAM, 1940).

Nas últimas décadas têm surgido "novas beta-lactamases", sendo que, atualmente, mais de 340 tipos são conhecidas (SHAH *ET AL.*,2004). Entre os vários tipos encontrados em enterobactérias, as enzimas derivadas de TEM e SHV, são as mais freqüentes. Mutações nos genes que codificam as beta-lactamases TEM e SHV podem expandir o espectro de atividade dessas enzimas, tornando-as capazes de hidrolisar penicilinas, cefalosporinas (exceto cefamicinas: cefoxitina e cefotetan), incluindo as de amplo espectro (ceftazidima, ceftriaxona, cefotaxima) e aztreonam sendo chamadas de beta-lactamases de espectro ampliado (ESBL). As ESBLs são predominantemente derivadas de TEM e SHV; no entanto, algumas enzimas do tipo OXA e CTX-M também mostram atividade contra esses antimicrobianos (BRADFORD, 2001).

1.3 ANTIMICROBIANOS BETA-LACTÂMICOS

Os beta-lactâmicos representam a classe mais variada e amplamente utilizada de antimicrobianos. A estrutura básica (Fig. 01) consiste um anel de tiazolidina (B) conectado a um anel beta-lactâmico (A) que se liga a uma cadeia lateral (R). Este grupo que inclui penicilinas, cefalosporinas, monobactâmicos e carbapenêmicos é responsável por aproximadamente 50% dos antimicrobianos utilizados de forma sistêmica, devido principalmente à sua baixa toxicidade e a grande variedade de compostos disponíveis.

A classificação mais utilizada é baseada nas diferenças estruturais do antimicrobiano; porém, subdivisões baseadas no espectro de atividade e na estabilidade dos compostos frente aos mecanismos de resistência bacteriana vêm sendo utilizadas (LIVERMORE, 1991). De acordo com a estrutura e espectro de atividade as penicilinas com ação contra Gram -negativos são divididas em aminopenicilinas (e.g. ampicilina), carboxipenicilinas (e.g. carbenicilina) e ureidopenicilinas (e.g. piperacilina). As cefalosporinas são divididas em cefalosporinas de primeira (e.g. cefalotina); segunda (e.g. cefoxitina), terceira (e.g. ceftazidima, cefotaxima e ceftriaxona) e quarta geração (e.g. cefepime). Outros grupos de antibióticos beta-lactâmicos são os monobactâmicos e carbapenêmicos (LIVERMORE, 2001).

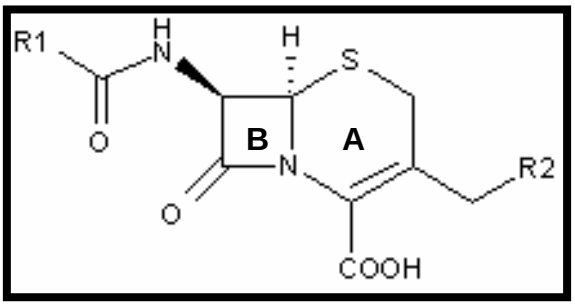

Figura 1 – Estrutura plana do antibiótico beta-lactâmico.
(www.google.com.br)

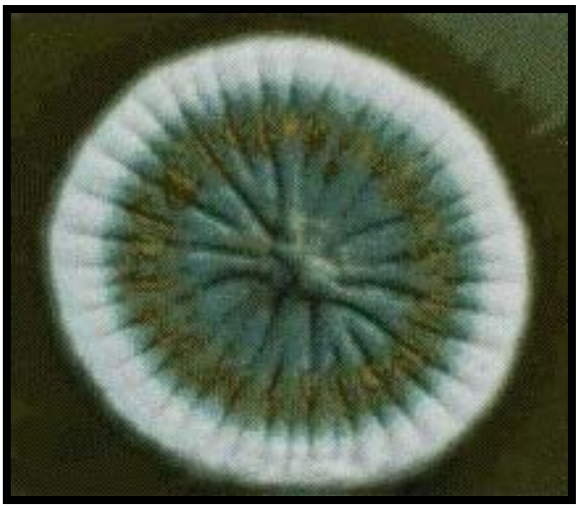

Figura 2 – Colônia de *Penicillium* sp.
(www.google.com.br)

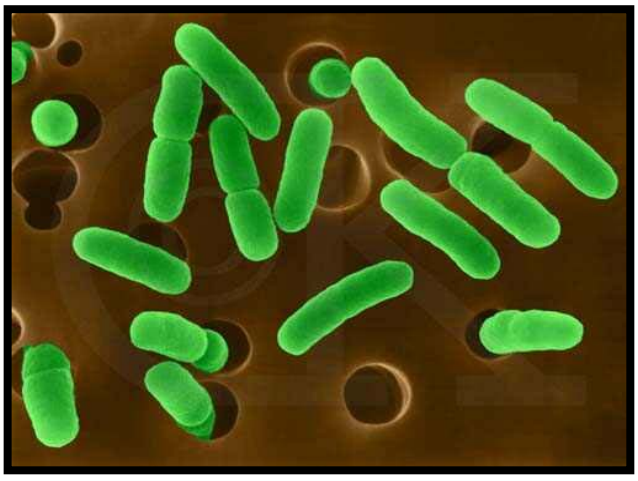

Figura 3 – Bacilos de *Escherichia coli.*
(www.google.com.br)

Os beta-lactâmicos são úteis e prescritos com freqüência na prática terapêutica, inibindo a síntese da parede celular bacteriana formada de peptídeoglicano. As cefalosporinas de terceira e quarta-gerações, monobactamicos e carbapenemicos são os beta-lactâmicos mais utilizados no tratamento de infecções causadas por enterobactérias (Menezes et al., 2000).

Portanto, uma maneira de impedir a ação destes antimicrobianos é a inativação destas drogas através de enzimas produzidas por estes microrganismos. Conseqüentemente, a sua atividade terapêutica torna-se ineficaz e a bactéria não é eliminada no local da infecção (SOUSA Jr., 2003).

Em geral, nas bactérias gram-positivas, estas enzimas são secretadas no meio extracelular. Dessa forma irão demonstrar uma atividade menor que as enzimas produzidas pelas bactérias gram-negativas, pois nestas, as beta-lactamases encontram-se estrategicamente situadas no espaço periplasmático, podendo alcançar maiores concentrações e agirem de modo mais eficiente sobre o anel beta-lactâmico. Esta inativação provoca a abertura do anel por hidrólise, transformando o antibiótico em um produto inativo (TAVARES, 1993; SZABÓ, 1997). Esta ação catalítica das beta-lactamases frente aos antimicrobianos é o principal mecanismo molecular que confere resistência das bactérias gram-negativas.

1.4 RESISTÊNCIA ANTIMICROBIANA

De acordo com os trabalhos mostrados por Rodrigues *et al* (2003) e Horta (2003), a resistência bacteriana está sendo alvo de várias pesquisas, tanto em amostras hospitalares como nas comunitárias, onde bactérias multi-resistentes encontram um campo fértil para a sua disseminação.

As enterobactérias são de grande importância no ambiente hospitalar, não apenas por seus fatores de virulência, mas porque podem apresentar resistência a várias classes de antimicrobianos. Estudos de vigilância de resistência aos antimicrobianos em ambiente hospitalar mostram que na América Latina a

resistência em gram-negativos é mais preocupante que em gram–positivos (SADER, 2000). Dentre os vários antimicrobianos usados na terapia contra Gram-negativos, os que apresentam índices mais críticos de resistência são os beta-lactâmicos, as quinolonas, o sulfametoxazol e os aminoglicosídeos. O que dificulta ainda mais a escolha da terapia adequada é o fato de muitas vezes a mesma bactéria apresentar vários mecanismos combinados, sendo resistente a quase todas as classes de antimicrobianos disponíveis (OPLUSTIL *ET AL*., 2001; GALES *ET AL*., 2002a; GALES *ET AL*., 2002b; SADER *ET AL*.,2003; SADER, 2000).

1.5 ENTEROBACTÉRIAS

A família *Enterobacteriaceae* envolve microrganismos ubíquos e constituintes da microbiota intestinal normal da maioria dos animais, incluindo seres humanos. Os membros dessa família são bacilos gram-negativos de tamanho médio (0,3 a 1,0 x 1,0 a 6,0 μm). Estes microrganismos são móveis dotados de flagelos peritríquios ou imóveis, não formam esporos e podem crescer rapidamente em condições aeróbias ou anaeróbias, em uma variedade de meios de cultura.

As enterobactérias possuem exigências nutricionais simples, fermentam glicose, reduzem o nitrato, são catalase-positivas e oxidase-negativas. A ausência da atividade de citocromo-oxidase constitui uma importante característica, visto que pode diferenciar as enterobactérias de outros bacilos Gram -negativos fermentadores ou não-fermentadores (O'HARA, 2005).

De todos os microrganismos envolvidos nos diversos mecanismos de penetração e infecção das vias urinárias, as bactérias de maior importância são as da família Enterobacteriaceae que aparecem com uma freqüência considerável dentro de ambientes nosocomiais. Elas produzem uma grande variabilidade de doenças humanas incluindo 30 a 35% de todos os casos de septicemia, mais de 70% das infecções das vias urinárias e muitas infecções intestinais (CASTRO, 2003 CARSON E NARBER, 2004; TRAUTNER E DAUROWCH, 2004).

1.5.1 *Klebsiella pneumoniae*

Konemam *et al* (1997) descreve que o gênero *Klebsiella* recebeu esta designação em homenagem a Edwin Klebs, um microbiologista alemão falecido no século XIX. Descrita por Carl Friedlander e durante muitos anos era conhecida como grande causadora de pneumonia severa, muitas vezes até fatal. As bactérias do seu gênero são bacilos Gram-negativos, saprófitas humanos, colonizando a nasofaringe e o trato gastrintestinal.

Características em meio de cultura revelam um crescimento mucóide muito viscoso, grandes cápsulas polissacarídicas com diâmetro de 3 a 4 mm e ausência de motilidade (Fig 04). De uma maneira geral, em reações bioquímicas estas bactérias produzem lisina por descaboxilação, metabolizam o citrato e revela reação positiva de Voges-Proskauer, além de fermentarem a lactose (KONEMAM, 1997).

1.5.2 *Escherichia coli*

Uma outra espécie bacteriana de extrema importância e a mais utilizada e estudada em pesquisas científicas atualmente é a *Escherichia coli* (Fig 05) (KI-SOO KIL, 1997). O gênero *Escherichia* assim denominado em homenagem a Theodor Von Escherich, que em 1885, descreveu a *Escherichia coli* (KONEMAM, 1997).

A *Escherichia coli* (Fig 05) é um bacilo Gram negativo e que produz em provas bioquímicas, reações positivas para: indol, descarboxilação da lisina e fermentação do açúcar manitol, além de produzir gás a partir da glicose. Os seus isolados em urocultura podem ser preliminarmente identificados pela capacidade de hemolisar em Ágar-Sangue e pela morfologia típica das colônias com "brilho" iridescente em meios diferenciais, como Ágar Teague, e pela positividade da reação do indol (KONEMAM, 1997).

Nas infecções adquiridas a *Escherichia coli* continua sendo a bactéria isolada com maior freqüência em amostras de sangue, feridas e principalmente no trato urinário, sendo a causa mais comum de infecção do trato urinário (ITU) a sua prevalência varia de 85% a 90%. (DE MOÜY, 1994; PHILIPPOM, 1996).

Já nas cidades de São Paulo e Belo Horizonte, trabalhos constataram que das espécies Gram-negativas mais isoladas de infecções hospitalares a *Escherichia coli* e *Klebsiella pneumoniae* foram as principais causadoras destas infecções (GALES *ET AL*., 1997; RESENDE *ET AL*., 1998). Nos Estados Unidos e Europa, estima-se que *Klebsiella* spp. seja responsável por 8% das infecções nosocomiais bacterianas, demonstrando que esta bactéria é um dos patógenos hospitalares mais importantes (SCHABERG *ET AL*., 1991; BERGOGNE-BEREZIN, 1995).

Estas espécies, presentes tanto nas infecções hospitalares como também nas infecções comunitárias, são produtoras de enzimas que conferem resistência antimicrobiana aos beta-lactâmicos denominadas de beta-lactamases. Tais enzimas catalisam a hidrólise do anel beta-lactâmico inativando estes antimicrobianos que impossibilitam uma terapêutica mais eficaz por apresentarem efeitos de resistência a estas drogas (KONEMAM, 1997).

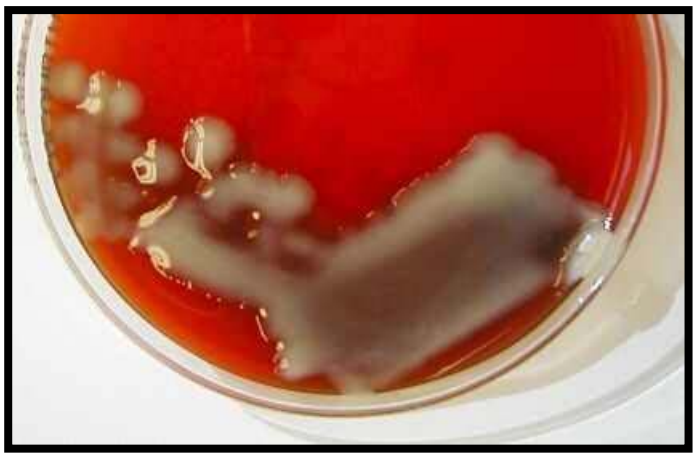

Figura 4 – *Klebsiella pneumoniae* em ágar sangue.
(Foto tirada pelo autor)

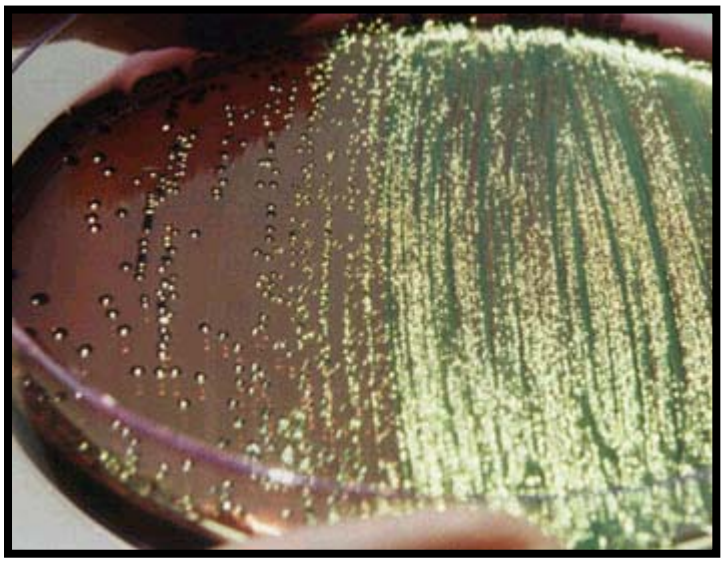

Figura 5 – *Escherichia coli*. em ágar teague
(Foto tirada pelo autor)

1.6 BETA LACTAMASES DE ESPECTRO ESTENDIDO (ESBL)

Com a introdução de novos beta-lactâmicos, observaram-se mudanças nas beta-lactamases. Estas mudanças foram responsáveis pela resistência às novas drogas como as cefalosporinas de largo espectro, carbapenêmicos e combinação de inibidores de beta-lactamases (PITOUT, 1997, DU BOIS *ET AL*., 1995;). Os estudos moleculares de tipagem plasmidial por Sader (1998), sugerem que a disseminação deste tipo de resistência é devida à seleção de cepas resistentes, ocasionadas pelo uso de antimicrobianos em hospitais. A ação hidrolítica das beta-lactamases pode ser bloqueada por inibidores de beta-lactamases, tais como: ácido clavulânico, sulbactam e tazobactam.

1.6.1 ESBL: Origem, Evolução e Enzimas Principais

As cepas produtoras de ESBL (beta-lactamases de espectro estendido) foram inicialmente identificadas na década de 80 na Alemanha por Knother (1983) e logo se tornaram prevalentes na Europa, disseminando-se para todo o mundo (JACOBY, 1991; PHILLIPON, 1989). Em 1985, o primeiro surto hospitalar causado por bactérias produtoras de ESBL ocorreu na França e depois nos EUA, no fim da década de 1980 e início da de 1990. O número de variantes de ESBL identificados tem crescido muito desde 1983, demonstrando a rápida evolução dessas enzimas. Mais de 100 variantes naturais são conhecidas até hoje, e esta lista continua a crescer (SHAH , 2004).

As enterobactérias produtoras de ESBL têm sido isoladas com bastante freqüência em pacientes com infecções do trato urinário provenientes de ambiente hospitalar (DARINI, 1995). Porém, podem ser encontradas em amostras de origem comunitária (SILVA, 2000, ARSLAN *ET AL*, 2005).

1.6.2 Tipos de ESBLs

Das classes importantes de beta-lactamases, as de espectro estendido são produzidas principalmente na família das *Enterobacteriaceae*. As ESBL são um grupo de enzimas de codificação plasmídica, derivadas das beta-lactamases clássicas (TEM-1, TEM-2 e SHV-1) e muitas ESBLs são mutantes destas beta-lactamases clássicas, com

um a quatro aminoácidos substituídos. Essas trocas correspondem a menos de 2% da seqüência da enzima, e são suficientes para remodelar o sítio ativo e torná-lo capaz de hidrolisar cefalosporinas de amplo espectro, tais como as cefalosporinas de 3ª e 4ª geração e também monobactâmicos (BRADFORD, 2001 MENEZES, 2000; THOMSON E MOLAND, 2001).

As pesquisas nos mostram, através de técnicas de biologia molecular, como por exemplo, a reação de polimerização em cadeia (PCR), que foram encontrados mais de 90 subtipos enzimáticos de beta-lactamases originados através de mutações da TEM e 25 subtipos destas enzimas originados da SHV (BRADFORD *ET AL.*, 1995). Elas são provenientes da pressão seletiva em ambientes onde há utilização abusiva de antimicrobianos de amplo espectro (KATSANIS *ET AL*,1995; JARNIER,1998). Existem outras classes de enzimas que já estão aparecendo em cepas resistentes, tais como o: CTX – M e a PER – 1 (COQUE *ET AL.*,2002).

1.6.2.1 ESBL do tipo TEM

A enzima TEM –1 (Fig 08), descrita no início dos anos 60, foi originariamente encontrada em uma cepa de *Escherichia coli* isolada de uma hemocultura em um paciente na Grécia de nome Temoniera, daí sua designação TEM (DATT E KONTOMICHALOU, 1965). A facilidade de transmissão mediada por plasmídeos e transposons facilitou a disseminação da TEM-1 para outras espécies de bactérias. Depois de alguns anos do isolamento da beta-Lactamase TEM-1, ela já havia sido encontrada não só na família *Enterobacteriaceae*, mas em vários gêneros de diferentes de bactérias, tais como: *Pseudomonas aeruginosa*, *Haemophilus influenzae* e *Neisseria gonorrhoeae*. A TEM-1 é capaz de hidrolizar penicilinas e cefalosporinas tais como: cefalotina e cefaloridina (BARTHÉLÉMY, 1985).

A enzima TEM-2, a primeira derivada da TEM-1, teve uma única mudança onde o aminoácido glicina foi substituído pela lisina na posição 39 (Gly39Lys). Na enzima TEM-17 a lisina substitui glutamato na posição 104 (Lys104Glu) (BLAZQUEZ *et al.*, 1995). Estas substituições nos aminoácidos que ocorrem dentro da própria enzima TEM podem ocorrer em um número ilimitado de posições, formando combinações que

resultam em várias alterações sutis nos fenótipos de ESBL (BARTHÉLÉMY, 1985; JACOBY, 1991; BREDFORD *ET AL*.,1995).

1.6.2.2 ESBL do tipo SHV

Outra Beta-Lactamase muito comum mediada por plasmídeos é a SHV-1 (Fig 07), presente em grande numero nas cepas de *Klebsiella pneumoniae*, mas podem ser encontradas em *Escherichia coli*. Esta enzima é responsável por 20% das transmissões plasmidiais que conferem resistência aos beta-lactâmicos de espectro estendido. Ao contrário das enzimas tipo – TEM, existem poucos derivados a SHV – 1. Provavelmente, isso acontece devido às poucas mudanças das posições dentro do gene estrutural. Algumas substituições são essenciais para a hidrólise de ceftazidima e outras para a hidrólise de cefotaxima (TZOUVELEKIS, 1999; BREDFORD *ET AL*., 1995).

A maioria de variantes da enzima SHV que possuem um fenótipo para ESBL é caracterizada pela substituição de glicina por serina na posição 238 (Gly238Ser). Muitas variantes também relacionadas a SHV-5 têm uma substituição de glutamato por lisina na posição 240 (Glu240Lys) na seqüência gênica. O resíduo de serina na posição 238 é crítico para a hidrólise eficiente de ceftazidima, e o resíduo de lisina é crítico para a hidrólise eficiente de cefotaxina (HULETSKY, 1993). A maioria das enzimas SHV são encontradas em várias cepas de *Klebsiella pneumoniae*. Porém, estas enzimas foram encontradas também em cepas de *Citrobacter diversus*, *Escherichia coli* e *Pseudomonas aeruginosa* (RASHEED, 1997; NAAS, 1999)

1.6.2.3 ESBL do tipo CTX

Em 1986, no Japão, Matsumoto *et al* (1988) descobriram uma nova enzima ESBL não-SHV, não-TEM, designada FEC-1 em um isolado de *Escherichia coli* resistente a cefotaxima de uma amostra da flora fecal em um laboratório canino que,

posteriormente, foram realizadas estudos farmacocinéticos frente aos antibióticos beta lactâmicos.

No final da década de 80, na Alemanha, Bauernfeind *et al* (1990) reportaram que em um isolado clinico de *Escherichia coli* resistente a cefotaxima não produtores de TEM e de SHV, uma nova enzima ESBL designada de CTX-M-1 (Fig 08), em referência a atividade hidrolítica contra a cefotaxima. Ao mesmo tempo, houve um aparecimento de cepas de *Salmonella* resistentes a cefotaxima na América do Sul mas também foram descritas em outras espécies da família das *Enterobacteriacea* (BAUERNFEIND *ET AL*, 1992).

Estas ESBLs são responsáveis pelo alto nível de resistência tanto a cefotaxima quanto a ceftazidima observada nestas regiões, mostrando uma grande atividade hidrolítica frente a estas drogas. Elas incluem as enzimas de CTX-M-tipo, CTX-M-1 (antigamente chamado MEM-1), CTX-M-2 e CTX-M-10 (BONNET, 2000).

As ESBLs da família CTX-M contêm 291 resíduos de aminoácidos, na qual confere uma massa molecular de aproximadamente de 28 kDa. (BONNET, 2004). Tudo indica que os aminoácidos glicina e prolina nas posições 240 e 167 estão envolvidos na evolução das enzimas do tipo CTX-M. As CTX-M-15, CTX-M-27, e CTX-M-16, que derivam da CTX-M-3, CTX-M-9, e CTX-M-14, respectivamente, tem uma substituição de glicina pelo ácido aspártico na posição 240 (Gly240Asp) que conferem um eficiente efeito catalítico contra a ceftazidima (BONNET *ET AL*, 2001; BONNET *ET AL*, 2003) A CTX-M-19, que deriva da CTX-M-18 tem uma substituição da prolina pela serina na posição 167, é uma CTX-M atípica porque possui um baixo K_M contra a ceftazidima do que contra a cefotaxima (BONNET, 2004)

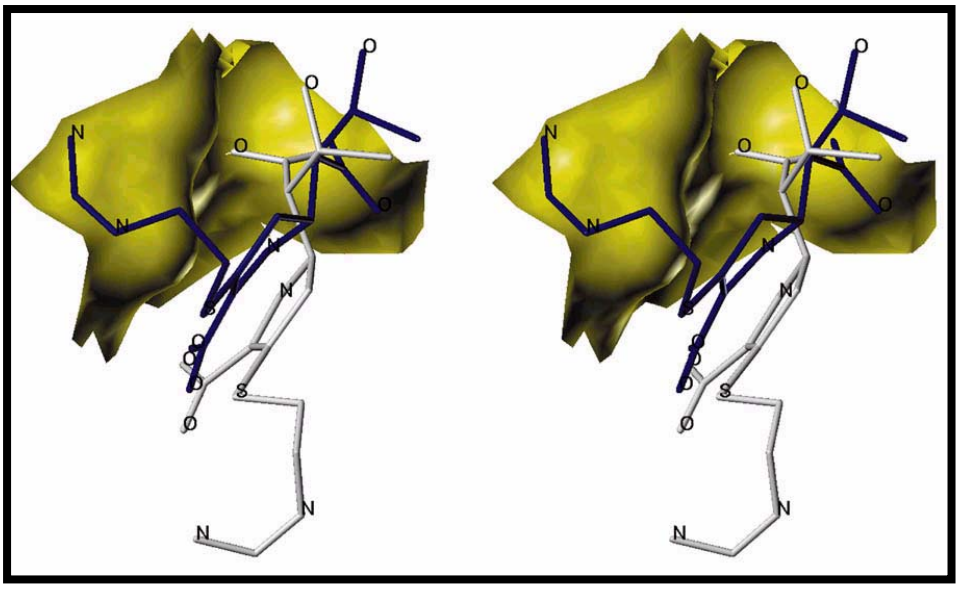

Figura 6 – Estrutura espacial da enzima TEM.
(http://www.ncbi.nlm.nih.gov)

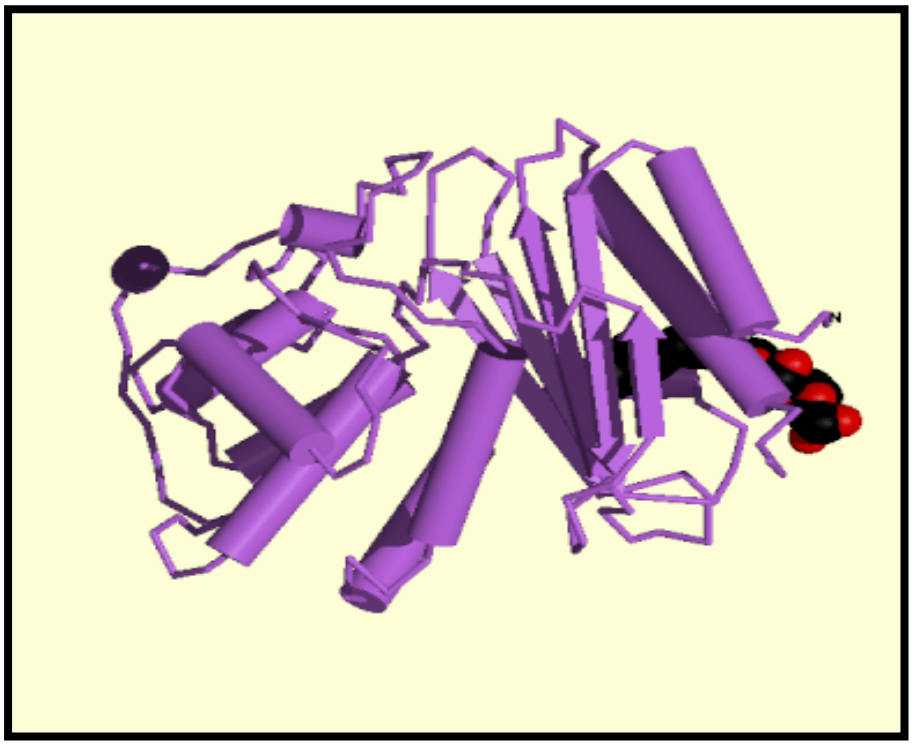

Figura 7 – Estrutura espacial da enzima SHV.
(http://www.ncbi.nlm.nih.gov)

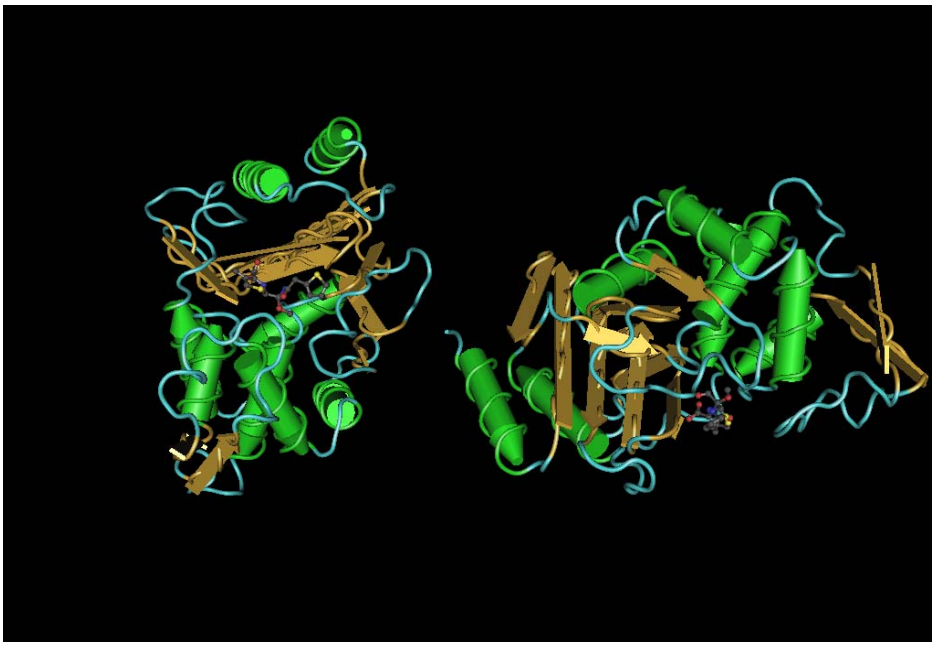

Figura 8 – Estruturas espacial da enzima CTX.
(http://www.ncbi.nlm.nih.gov)

1.7 EPIDEMIOLOGIA

O estudo da evolução biológica das bactérias é necessário para a identificação e monitoramento da sensibilidade aos diversos antibióticos utilizados nos serviços de rotina de diagnostico bacteriológico. Conforme o trabalho realizado por Hsiung (2001), as bactérias adquirem resistência aos beta-lactâmicos de espectro ampliado por causa do uso indevido e abusivo de antibióticos.

As bactérias produtoras de ESBL encontram-se espalhadas pelo mundo, todas com alta incidência na Europa, sendo que 32,8% das amostras de *Klebsiella* spp. e 14,4 % das amostras de *Escherichia coli* isoladas nessa região, produzem ESBL (JONES *ET AL*., 2003). Um estudo realizado na Espanha detectou cepas produtoras de ESBL em 90% dos hospitais participantes de um program a de vigilância (HERNANDEZ *ET AL*., 2003). Vários relatos da produção de ESBL ocorreram também na Itália (PAGANI *ET AL*., 2003; PAGANI *ET AL*., 2002), França (BERTRAND *ET AL*., 2003; GALDBART *ET AL*., 2000; MHAND *ET AL*.,1999; CHAMPS *ET AL*.,2000) e Polônia (GNIADKOWSKI *ET AL*.,1998). Na Ásia várias ocorrências foram descritas em Taiwan (WU *ET Al*., 2003), Israel (NAVON-VENEZIA *ET AL*., 2003) e Korea (KIM *ET AL*., 1998; PAI *ET AL*., 2001; PAI *ET AL*., 1999). E ainda: EUA (SCHAWBER *ET AL*., 2004), Canadá (MULVEY *ET AL*., 2003) e México (SILVA *ET AL*., 2001), entre outros.

As cepas produtoras de ESBL disseminaram-se rapidamente por todo o mundo e, quando estabelecidas em uma região, freqüentemente passam a ser o mecanismo de resistência prevalente. A literatura demonstra muitos casos onde hospitais têm notado rápido aumento no número de microrganismos carreando ESBL, além de disseminação intra-hospitalar ou entre hospitais vizinhos (WINOKUR *ET AL*., 2000). A transmissão pelas mãos dos profissionais é relevante, sendo o trato gastrintestinal dos pacientes um importante reservatório (BRITO *ET AL*., 1999; WINOKUR *ET AL*., 2000). Alguns surtos foram resultantes de contaminação de aparelhos e insumos diagnósticos, como termômetros e gel usado em ultra-sonografia (GNIADKOWSKI, 2001).

Além da transmissão das cepas resistentes ocorre também a aquisição de resistência por cepas susceptíveis (BERTRAND *ET AL*., 2003; SILVA *ET AL*., 2001; RAHAL, 2000). A transmissão horizontal do gene de resistência é facilitada por estar freqüentemente codificado em plasmídeos (WU *ET AL*., 2003; MHAND *ET AL*., 1999), que podem ser facilmente transferidos entre as cepas (PAGANI *ET AL*., 2003; GALDBART *ET AL*., 2000; MULVEY *ET AL*., 2003; PAI *ET AL*., 1999; GALANI, 2002; SZABÓ *ET AL*., 1999).

Em virtude das dificuldades técnicas de detecção, a prevalência de beta-lactamases de espectro estendido é geralmente subnotificada e permanece desconhecida na maioria dos laboratórios de rotina. Estas técnicas possuem algumas limitações, tais como: a hiperprodução de algumas beta-lactamases cromossômicas e o alto custo das técnicas de biologia molecular. Desse modo, a falha na identificação de ESBL através de testes de rotina pode levar a uma utilização inapropriada de cefalosporinas de 3ª geração com conseqüente aumento de mortalidade (CASTRO, 2003; HORTA, 2003).

A pesquisa dentro do laboratório é imprescindível na detecção das enterobactérias produtoras de ESBL e de possíveis surtos nosocomiais. A detecção precoce destas cepas é extremamente importante para instaurar o tratamento adequado e as medidas de isolamento aos pacientes necessários para se evitar a sua disseminação promovendo condutas preventivas de saúde publica (SOUSA, 2003; MENEZES, 2003).

O estudo epidemiológico dos microrganismos causadores de patologias, principalmente, nas vias urinárias produtores de beta-lactamases de espectro estendido (ESBL) e o estabelecimento dos perfis de sensibilidade e molecular frente aos antimicrobianos são aspectos de grande importância para o laboratório.

A sua investigação propiciará identificação da presença de cepas multi-resistentes e dos genes de ESBL envolvidos na resistência a antimicrobianos, fornecendo subsídios para um melhor conhecimento dos determinantes gênicos dos padrões de resistência das bactérias isoladas proporcionando maiores chances de sucesso na conduta terapêutica.

2.0 OBJETIVOS

2.1 OBJETIVO GERAL

Investigar a produção de ESBL e os genes SHV, CTX e TEM em isolados de *Klebsiella pneumoniae* e *Escherichia coli* em portadores de infecções de trato urinário em pacientes do Hospital Universitário Oswaldo Cruz.

2.2 OBJETIVOS ESPECÍFICOS

- Investigar o perfil de resistência de isolados nas duas espécies de bactérias;
- Comparar a ocorrência de produção de ESBL nestas duas espécies
- Determinar a presença dos genes SHV, CTX e TEM nos isolados das duas espécies de bactérias;

3. REFERÊNCIAS BIBLIOGRÁFICAS

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SHV, TEM and CTX-type extended spectrum beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* isolates from nosocomial and community-acquired urinary tract infections

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Abstract

Extended spectrum beta-lactamases are already wideworld spreaded, conferring clinical important multi-resistance phenotypes to bacteria harboring those enzymes. Patients with UTIs are mainly infected with *E. coli* and *K. pneumoniae*, the most frequent ESBL producing strains. In this work, we analysed the presence of ESBL producing strains of *E. coli* and *K. pneumoniae* isolated from nosocomial and community- acquired UTIs. Susceptibility patterns and the presence of ESBL genes SHV, TEM and CTX were studied in ESBL positive strains. The results showed that ESBL producing strains were present in 25% of nosocomial and 9.3% of community-acquired UTIs. ESBLs was produced by 12.2% and 18.2 % of *E. coli* and *K. pneumoniae* isolates, respectively. ESBL genes were detected in both nosocomial and community-acquired isolates. All the isolates carrying multiple ESBL genes showed multi-drug resistance phenotypes, which alerts to the necessity of continuous surveillance programs in UTIs.

Key words: ESBL, urinary tract infections, *E.coli, K. pneumoniae*

Introduction

Urinary tract infections (UTIs) are one of the most common infections in both hospital and community environments. Main predictable ethiologic agents related to these infections are *Escherichia coli* and *Klebsiella pneumoniae*. One important concern to these species is the increasing world-wide detection of broad spectrum mechanisms of resistance to beta lactams [1, 2, 3]. Beta lactam agents are the most frequently prescribed antibiotics [1, 4, 5] and the production of different beta lactamases by clinical isolates has been continuosly described in the literature [6, 7]. In such context, many new beta lactamases belong to ESBL group (extended spectrum beta lactamases), enzymes capable of hydrolyse oxyimino-cephalosporins and monobactams, but not cephamycins or carbapenems [1, 8, 9]. ESBL are predominantly identified in *E. coli* and *K. pneumoniae* and ESBL bacteria causing urinary tract infections are clinically relevant due to the restricted options in therapeutic procedures and possibility of failures in empiric prescriptions. ESBLs derived from mutations in TEM and SHV beta lactamases, originating the derivatives TEM and SHV- type ESBLs. However, many other ESBLs have been already described, as CTX, OXA, PER and GES [8, 10]. In Enterobacteriaceas, the most common ESBLs are SHV, TEM and CTX-types [8, 11]. Most of the UTIs caused by ESBL-producing organisms have been reported from inpatients. However, in the last years, reports on ESBL positive strains from community-acquired UTIs had been relevant [12, 13], perhaps due to the carriage by outpatients to community or by multiple courses of antibiotics in the community [14]. One important concern to the fact that usually ESBL genes are present in large plasmids, most of them carring resistance to other antimicrobial agents, such as aminoglycosides, trimethoprim, sulphonamides and chloramphenicol. Thus, the spread of such plasmids could rapidly disseminate multi-

drug resistance strains in the community. In this study, we describe the occurrence of ESBL-producing uropathogens *E.coli* and *K. pneumoniae* in nosocomial and community- acquired UTIs, as well ''describing their associated resistances.

Materials and Methods

Bacterial strains

Consecutive non-duplicated isolates of *E. coli* (43) and *K. pneumoniae* (20) were collected from community-acquired and nosocomial urinary tract infections from inpatients and outpatients of the Oswaldo Cruz University Hospital, Recife, Brazil. Community-acquired criteria was applied to those patients who had not been hospitalized in the last thirty days.

Antimicrobial susceptibility tests

Antimicrobial susceptibilities were determined by disc diffusion method, according to the recommendations of NCCLS [15]. ESBL production was tested by the double disc synergy (DDS) method, using ceftazidime (30μg), cefotaxime (30μg), ceftazidime (30μg) – clavulanic acid (10μg) and cefotaxime (30μg) - clavulanic acid (10 μg) discs, as recommended by NCCLS [15].

Molecular detection of ESBL genes

Primers used in this study are listed in Table 1. DNAs from bacterial strains were used as template for PCR reactions. PCR products were analyzed in 0.9% agarose gels by ethidium bromide staining.

Results and Discussion

Not much studies on ESBL producing organisms which cause UTIs are available from Brazil, especially in community-acquired infections. This study investigated the occurrence of ESBL-producing pathogens among nosocomial and community-acquired UTIs. A total of 43 community-acquired and 20 nosocomial urinary tract infections isolates were evalualed tested by ESBL DDS test. Community-acquired and nosocomial *E. coli* counted to 33 and 9, respectively. For *K. pneumoniae*, the isolates counted to 11 in community-acquired and 11 in nosocomial UTIs. DDS positive results were detected in 14.3% (9/63) of the isolates which were, thus, found in both groups nosocomial (25%) and community-acquired (9.3%) UTIs. All community-acquired DDS positive isolates were from *E. coli*. Therefore confirming this species as the major community-acquired uropathogens [14]. Its is a noteworthy find the presence of ESBL positive isolates among community-acquired UTIs. Although such results are still little reported in the literature, ESBL-producing uropathogens isolated from community-acquired UTIs had been already described [16]. For *E. coli*, Kahlmeter et al. [14] reported a significant correlation between the degree of antimicrobial consumption and the incidence of multi-drug resistant isolates in community-acquired UTIs, which could contribute to the

development of resistance to broad spectrum cephalosporins in the literature. In nosocomial DDS positive cases, *K. pneumoniae* and *E. coli* counted to 4 and 1 isolates respectively. Our results agree with previous studies from Asia and Latin-America that reported high prevalence rates ESBL-producing strains of *K. pneumoniae* from nosocomial urinary tract [17, 18, 19]. ESBL producers were tested for the presence of TEM, CTX and SHV derivatives in order to investigate the dissemination of those enzymes in the microbial population studied, a representative gel is shown in figure 1. Genes encoding TEM-type were detected in 3 of 5 (60%) *E. coli* and in 2 of 4 (50%) *K. pneumoniae*. SHV-type were detected only in *E. coli* isolates, 3 of 5 (60%) and the CTX-type were present in 1 of 4 (25%) *K. pneumoniae* isolates and in 1 of 5 (20%) *E. coli*. Multiple ESBL genes were detected in one isolate of *K. pneumoniae* (CTX, TEM) and two isolates of *E. coli* (TEM, SHV, CTX) the later being the only nosocomial *E. coli* isolate. These findings showed the presence of all the three most common ESBL genes in the microbial population investigated, including the community-acquired uropathogens the most prevalent types were TEM and SHV, as also observed by Ryoo et al. [20]. No detection of SHV-type ESBL in *K. pneumoniae* isolates could be due to the presence of other ESBL genes in such nosocomial isolates which were not investigated in the present work. The ESBL producing isolates showed high rates of resistance toward most of the commonly prescribe drugs for UTIs. In this work, resistance to nalidixic acid, nitrofurantoin, ciprofloxacin, gentamicin, sulfamethoxazol – trimethoprim and chloranphenicol were verifed (table 2). *E. coli* isolates were particularly highly resistance to trimethoprim-sulfamethoxazol, nalidixic acid, ciprofloxacin and chloramphenicol. *K. pneumoniae* isolates showed higer resistance rates to trimethoprim-sulfamethoxazol, nalidixic acid and nitrofurantoin. Its of great

concern the finding that all the isolates carrying multiple ESBL genes showed multi-drug resistance phenotype, possibly due to the association of antibiotic resistance genes in large plasmids [16]. The present data showed that ESBL production was high among the uropathogens studied, although the size of the population investigated must be increased. However, the observations on the occurrence of ESBL genes in the population are very important, especially in community-acquired uropathogens, since they can encourage the rational use of antimicrobial drugs and alert to the necessity of a continuous surveillance on community-acquired UTIs.

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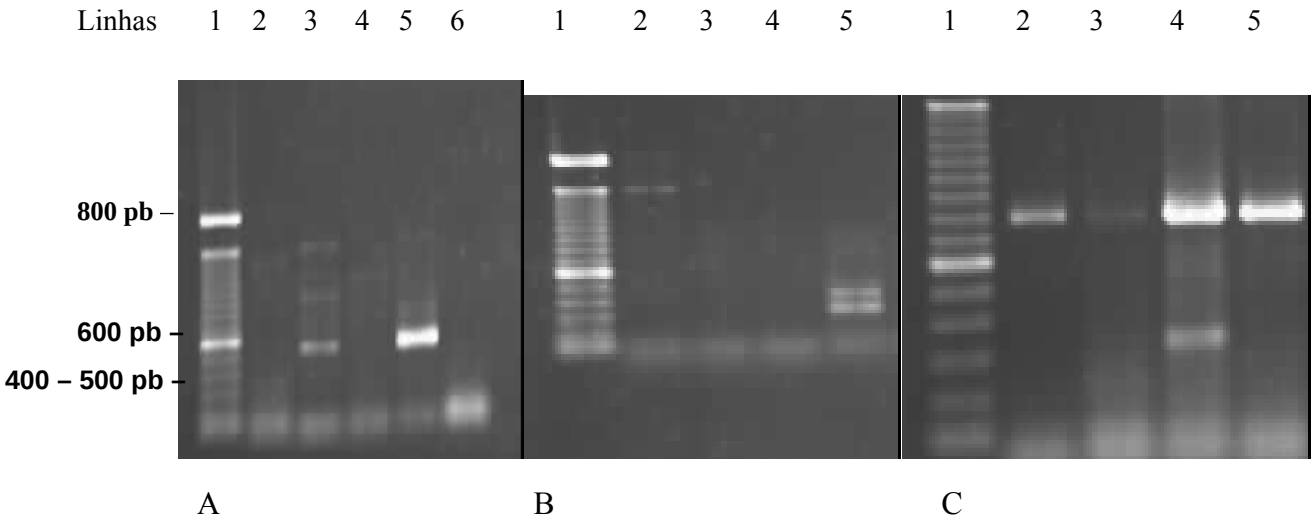
Table 1 – Primers used for ESBL genes detection.

PCR target	Prime name	Oligonucleotide sequence (5' – 3')
bla $_{SHV}$	SHV - F	ATGCGTTATATTCGCCTGTG
	SHV – R	TTAGCGTTGCCAGTGCTCGA
bla $_{TEM}$	TEM – F	TTTTTTGCGGCATTTTGC
	TEM – R	TGTCTATTTCGTTCATCCA
bla $_{CTX}$	CTX – F	ATGGTTAAAAAATCACTGCGCC
	CTX – R	TTACAAACCGTCGGTGACGA

Table 2 – Percentual of resistance showed by ESBL producing uropathogens against antibiotics usually prescribed for UTI

	E. coli		*K. pneumoniae*	
Antimicrobial Agents	Resist (%)	N	Resist (%)	N
Nalidixic Acid	60	5	100	2
Nitrofurantoin	40	5	100	2
Ciprofoxacin	50	4	50	4
Gentamicin	0	2	33	4
Sulfamethoxazole-trimethoprim	100	3	100	4
Chloramphenicol	50	4	75	4

Figure 1. Representative gel showing CTX (A). SHV (B) AND TEM (C) genes detection in urinary tract isolates. CTX, SHV and TEM amplicons showed sizes of 600, 400-500 and 800 bp, respectively. A) Line1: ladder; lines2,4,6: clinical isolates negative for CTX genes; lines 3, 5; clinical isolates positive for CTX genes; B) Line1: ladder; lines 2, 3, 4: clinical isolates negative for SHV genes; line 5; clinical isolate positive for SHV genes; C) Line1: ladder; line3: clinical isolate negative for TEM genes; lines2, 4, 5; clinical isolates positive for TEM genes.


Linhas 1 2 3 4 5 6 1 2 3 4 5 1 2 3 4 5
800 pb –
600 pb –
400 – 500 pb –
A
B
C

5. CONCLUSÕES

5. CONCLUSÕES

1. Nos casos de infecção urinária estudadas, houve uma ocorrência significante de patógenos produtores de beta lactamases de espectro estendido;
2. Nas infecções urinárias de comunidade, houve uma importante detecção de patógenos produtores de ESBL;
3. Na população investigada, houve uma presença dos três genes de ESBL, principalmente as ESBLs do tipo TEM e do tipo SHV, incluindo nas infecções urinárias em comunidade;
4. Os isolados produtores de ESBL mostraram-se resistentes aos antimicrobianos mais comuns na prática terapêutica. Foi verificado que tanto os isolados de *E. coli* e *K. pneumoniae* apresentaram uma alta resistência aos antimicrobianos.

6. APÊNDICE

6.1. FICHA DE ACOMPANHAMENTO

FICHA DE ACOMPANHAMENTO – ISOLADO BACTERIANO EM INFECÇÕES URINÁRIAS

Nº.

ISOLADO DE: _____ LAB. BACTERIOLOGIA – HUOC
DATA DO ISOLAMENTO: ___/___/_____ DATA DA INTERNAÇÃO: ___/___/_____
PACIENTE:
_____
PRONTUÁRIO Nº.: _____ REGISTRO LABORATÓRIO: _____
LOCAL: _____ IDADE: _____ SEXO: _____
USO PRÉVIO DE ANTIBIÓTICO? SIM ( ) NÃO ( ) QUAL?_____
INFECÇÃO NOSOCOMIAL? SIM ( ) NÃO ( )
O PACIENTE FOI A ÓBITO? SIM ( ) NÃO ( ) QUANDO? ___/___/_____
O PACIENTE RECEBEU ALTA? SIM ( ) NÃO ( ) QUANDO? ___/___/_____

ANTIBIOGRAMA

Nº.	ANTIBIÓTICO		Nº.	ANTIBIÓTICO	
1	Ácido pipemídico		16	Cloranfenicol	
2	Ácido nalidíxico		17	Clindamicina	
3	Nitrofurantoína		18	Pemicilina G	
4	Norfloxacina		19	Oxacilina	
5	Ciprofloxacina		20	Eritromicina	
6	Moxifloxacina		21	Imipenem	
7	Gentamicina		22	Meropenem	
8	Amicacina		23	Teicoplasmina	
9	Amoxicilina + SV3		24	Vancomicina	
10	Ampicilina		25	Sulfa-trim	
11	Piperacilina+Tazo		26	Tetraciclina	
12	Cefalotina		27	Linezolida	
13	Cefoxitina		28	Aztreonam	
14	Cefotaxina		29	Ertapenem	
15	Cefedime		30	Polimixina B	

Legenda: (R) – Resistente; (S) – Sensível; ESBL: Positiva ( )
(X) – Não testado Negativa ( )

OBSERVAÇÕES_____
_____
_____

7. ANEXOS

6.1 NORMAS PARA PUBLICAÇÃO DA REVISTA JOURNAL ANTIMICROBIAL CHEMOTHERAPY

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Reeves DS, Wise R, Drummond CWE. Duplicate publication: a cautionary tale. *J Antimicrob Chemother* 2004; **53**: 411-2.

Supplementary data

Please note that it is also possible to include files containing supplementary data. The supplementary data (for example large tables of MICs, or a questionnaire) can be lodged with the version of the paper published online as an extra resource for readers. Please contact the Editorial Office if you would like further details.

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All documents should be double spaced, with wide margins. A clear, legible single font (which is readily available internationally) and point size should be employed throughout. For symbols, please use the 'insert symbol' function and ONLY select characters from the 'normal text' subset. **All submitted articles should be line numbered (using continuous line numbers). To do this in Word, use File, Page Setup, Layout, Line Numbers and select continuous line numbering. Please DO NOT insert page numbers (as the pdf proof created by the online submission system will automatically be page numbered).**

Original articles and Brief reports must have a structured synopsis. The headings for the structured synopsis are as follows: Objectives, Patients and methods (or Methods), Results, and Conclusions. Authors should also provide 3-5 keywords. Very general terms such as 'bacteria' and 'human' and terms already present in the title should be avoided, as should non-standard abbreviations.

Original articles. There is no length limit for this format; however, papers must be written as concisely as possible. Original articles are divided into the following sections: Synopsis (250 words maximum), Introduction, Materials (or Patients) and methods, Results, Discussion, Acknowledgements and References. Repetition of content between sections must be avoided. A combined Results and Discussion section is acceptable.

Brief reports. These should have the same format as Original articles, but should have no more than two figures/tables, should have a maximum of 10 references and should not exceed 1500 words of text.

Antimicrobial practice. Short articles on topics related to the use of antimicrobials, format as for Brief reports.

Correspondence. Letters on topics of concern or interest in the field of antimicrobial chemotherapy, particularly arising from papers or letters already published in the Journal. These should be addressed to the Editor-in-Chief and must not exceed 800 words, one figure or table and six references.

Case reports. JAC will publish Case reports that are of sufficient calibre and potential importance, and they should be submitted in the form of Correspondence (see above).

Systematic review articles. There is no length limit for this format. They should be summaries of previously published literature that use explicit methods to identify, select and appraise relevant research, and that use appropriate statistical methods to combine the valid studies. They should include a structured synopsis (with appropriate headings; these may differ from the headings used for Original articles etc.).

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STYLE

General

In addition to reading the information provided here, authors should consult a recent issue of the Journal for the layout and conventions used.
The past tense should be used throughout for description of the results of the paper, the present tense should be used when referring to previously established and generally accepted results.
Where possible SI units should be used.

Spelling

British spelling should be used. Spelling should follow that of the *Oxford Dictionary for Scientific Writers and Editors* and where this gives no guidance the *Concise Oxford Dictionary*. Spelling of drug names should conform with that given in the latest edition of the *British National Formulary* (published by the British Medical Association and the Royal Pharmaceutical Society of Great Britain and available online at http://www.bnf.org/webnf/lform1/bnf/index.html).

Abbreviations

Non-standard abbreviations should be defined at the first occurrence and introduced only where multiple use is made. The following abbreviations may be used without definition:

Organizations

- ATCC American Type Culture Collection
- CDC Centers for Disease Control (and Prevention) (USA)
- CDSC Communicable Disease Surveillance Centre (UK)
- HPA Health Protection Agency (UK)
- MRC Medical Research Council (UK)
- NCCLS National Committee for Clinical Laboratory Standards (as of 2005: CLSI Clinical and Laboratory Standards Institute)
- NIH National Institutes of Health (USA)
- WHO World Health Organization

General

- AIDS acquired immunodeficiency syndrome
- AMP, ADP, ATP etc. adenosine 5'-phosphate, adenosine 5'-diphosphate, adenosine 5'-triphosphate
- bp base pair(s)
- BSA bovine serum albumin
- cfu colony-forming unit(s)
- CNS central nervous system
- cpm counts per min
- CSF cerebrospinal fluid
- Da dalton(s)
- DNA, cDNA deoxyribonucleic acid, complementary DNA
- dpm disintegrations per min
- ELISA enzyme-linked immunosorbent assay
- **g** acceleration due to gravity
- h hour(s)
- HEPES 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid
- HPLC high-performance (or high-pressure) liquid chromatography

- ID_{50} 50% infective dose
- IU international unit(s)
- kg kilogram(s)
- L litre(s)
- LD_{50} 50% lethal dose
- m metre(s)
- MBC minimum bactericidal concentration
- MIC minimum inhibitory concentration
- min minute(s)
- MLD minimum lethal dose
- mol. wt molecular weight
- MOPS 4-morpholinepropanesulphonic acid
- nt nucleotide(s)
- *P* probability
- PAGE polyacrylamide gel electrophoresis
- PBS phosphate-buffered saline
- PCR polymerase chain reaction
- PFGE pulsed-field gel electrophoresis
- pfu plaque-forming unit(s)
- ppm parts per million
- RNA ribonucleic acid
- rpm revolutions per min
- RT-PCR reverse transcriptase PCR
- s second(s)
- SDS sodium dodecyl sulphate
- sp./spp. species (singular)/species (plural)
- Tris 2-amino-2-hydroxymethylpropane-1,3-diol
- U unit(s)

Dosage and routes of administration

Dosage frequencies should be given in full in English at each occurrence. Abbreviations are not permitted. Routes of administration other than intramuscular (im) and intravenous (iv), which may be abbreviated after definition, should be given in full in English.

Pharmacokinetics

- AUC area under the concentration-time curve
- $AUC_{0\text{-}24}$ and $AUC_{0\text{-}\infty}$ AUC from 0-24 h and AUC from 0 h to infinity
- CL clearance
- CL_{CR} creatinine clearance
- CLN_R non-renal clearance
- CL_R renal clearance
- C_{max} maximum concentration of drug in serum
- k_{el} elimination rate constant
- k_{ss} residence rate constant at steady state
- $t_{½}$ half-life
- T_{max} time to maximum concentration of drug in serum
- *V* volume of distribution
- V_{max} maximum rate of metabolism
- V_{ss} volume of distribution at steady state

Pharmacodynamics

For acceptable abbreviations and their definitions please refer to the article by Mouton et al. [Mouton JW, Dudley MN, Cars O, Derendorf H & Drusano GL. Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs. *Int J Antimicrob Agents* 2002; **19**: 355-8.]

Antimicrobial agents

Abbreviations of antimicrobial agents are only permitted in Tables and Figures, provided that they are defined in the legends. Please use the following abbreviations:

Antibacterial agents

- amikacin AMK
- amoxicillin AMX
- ampicillin AMP
- ampicillin/sulbactam SAM
- azithromycin AZM
- azlocillin AZL
- aztreonam ATM
- carbenicillin CAR
- cefaclor CEC
- cefadroxil CFR
- cefalexin LEX
- cefalotin CEF
- cefamandole FAM
- cefapirin HAP
- cefazolin CFZ
- cefdinir CDR
- cefditoren CDN
- cefepime FEP
- cefetamet FET
- cefixime CFM
- cefmetazole CMZ
- cefonicid CID
- cefoperazone CFP
- cefotaxime CTX
- cefotetan CTT
- cefoxitin FOX
- cefpodoxime CPD
- cefprozil CPR
- cefradine RAD
- ceftazidime CAZ
- ceftibuten CTB
- ceftizoxime ZOX
- ceftriaxone CRO
- cefuroxime CXM
- chloramphenicol CHL
- cinoxacin CIN
- ciprofloxacin CIP
- clarithromycin CLR
- clinafloxacin CLX
- clindamycin CLI

- co-amoxiclav AMC
- daptomycin DAP
- dicloxacillin DCX
- dirithromycin DTM
- doxycycline DOX
- enoxacin ENX
- erythromycin ERY
- fleroxacin FLE
- fosfomycin FOF
- gatifloxacin GAT
- gentamicin GEN
- grepafloxacin GRX
- imipenem IPM
- kanamycin KAN
- levofloxacin LVX
- linezolid LZD
- lomefloxacin LOM
- loracarbef LOR
- meropenem MEM
- methicillin MET
- mezlocillin MEZ
- minocycline MIN
- moxalactam MOX
- moxifloxacin MXF
- nafcillin NAF
- nalidixic acid NAL
- netilmicin NET
- nitrofurantoin NIT
- norfloxacin NOR
- ofloxacin OFX
- oxacillin OXA
- penicillin PEN
- piperacillin PIP
- piperacillin/tazobactam TZP
- quinupristin/dalfopristin Q/D
- rifabutin RFB
- rifampicin RIF
- rifapentine RFP
- sparfloxacin SPX
- spectinomycin SPT
- streptomycin STR
- teicoplanin TEC
- telithromycin TEL
- tetracycline TET
- ticarcillin TIC
- ticarcillin/clavulanic acid TIM
- tobramycin TOB
- trimethoprim TMP
- trimethoprim/sulfamethoxazole SXT
- trovafloxacin TVA
- vancomycin VAN

β-lactamase inhibitors

- clavulanic acid CLA
- sulbactam SUL
- tazobactam TZB

Antifungal agents

- amphotericin B AMB
- clotrimazole CLT
- flucytosine 5FC
- fluconazole FLC
- itraconazole ITC
- ketoconazole KTC
- nystatin NYT
- terbinafine TRB
- voriconazole VRC

Antiviral agents

- aciclovir ACV
- cidofovir CDV
- didanosine ddI
- famciclovir FCV
- foscarnet FOS
- ganciclovir GCV
- indiavir IDV
- lamivudine 3TC
- penciclovir PCV
- ritonavir RTV
- saquinavir SQV
- stavudine d4T
- valaciclovir VCV
- zalcitabine ddC
- zidovudine ZDV

Bacterial nomenclature

When genus and species are given together use a capital letter for the genus and a lowercase letter for the species and italicize both e.g. *Staphylococcus aureus*. After the initial use in the text of the full name of an organism the generic name should then be abbreviated to the initial letter, e.g. *E. coli*.
When the genus is used as a noun or adjective use lowercase roman unless the genus is specifically referred to e.g. 'staphylococci and streptococci' but 'organisms of the genera *Staphylococcus* and *Streptococcus*'.
The name of an order has an initial capital but is not italicized, e.g. Enterobacteriaceae. For genera in the plural, use lowercase roman, e.g. salmonellae.
When the species is used alone use lowercase e.g. viridans streptococci. For trivial names, use lowercase roman e.g. meningococcus.
Authors should use bacterial names present in the *Approved List of Bacterial Names, Amended Edition* (1989), Skermanm, V.B.D, McGowan, V. & Sneath, P.H.A., Eds, ASM Press, Washington, DC, USA (ISBN 1-55581-014-4), with subsequent alterations validly published by announcement in Validation Lists of the *International Journal of Systematic and Environmental Microbiology* (formally the *International Journal of Systematic Bacteriology*). A full list of validly published bacterial names is given at http://www.bacterio.cict.fr/allnames.html

Genetic and amino acid nomenclature

Bacterial genetics. Genotype designations are indicated with italic lowercase three-letter locus codes (e.g. *par*, *his*, *ara*). If several loci are involved in a related function the individual loci are designated by the addition of an uppercase italic letter to the locus code (*parC*, *ompF*).
Phenotype designations (for example the protein product of a bacterial gene) are given in roman type with an initial capital letter (OmpF, LacZ).
Erythromycin gene nomenclature should follow that described in: Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J & Seppala H. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999; **43**: 2823-30.
Yeast genetics. Wild-type alleles are all uppercase and italicized (*LEU2*), mutant alleles are all lowercase and italicized (*leu2*), and gene products are capitalized on the first letter and are not italicized (Leu2).
General. Authors should ensure that they confine discussion of changes in amino acid sequence to the context of the protein (e.g. OmpF) and nucleotide changes to the context of the gene (e.g. *ompF*). Please also be aware of the difference between a mutant (a strain with one or more mutations) and a mutation (a change in the sequence of the genetic material).
Amino acids. The full residue names or three-letter abbreviations are preferred in the text (e.g. a methionine residue at position 184 should be symbolized Met-184 or Met^{184}). The single letter codes may be used in figures. Amino acid changes should be designated Met-184®Val, Met^{184}®Val or M184V.
When comparing nucleotide or amino acid sequences authors should exercise care in the use of the term homology. Homology should only be used when a common evolutionary origin is being implied; it is incorrect to give a percentage homology between two sequences. The wing of a bird and the human arm are homologous structures (they are believed to have a common evolutionary origin), homology cannot be quantified. For sequence comparison authors should use the terms identity and similarity. Sometimes 'equivalent' or 'counterpart' is more appropriate than 'homologue'.

FICI data

Fractional inhibitory concentration index (FICI) experiments are performed in order to study drug interactions and they must be interpreted in the following way:
FICI<=0.5 = synergy
FICI>4.0 = antagonism
FICI>0.5-4 = no interaction
For further information please see the following Editorial:
Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother* 2003; **52**: 1.

Chemistry

General nomenclature. The IUPAC recommendations on chemical nomenclature should be followed (*IUPAC Compendium of Chemical Terminology* (1987, ISBN 0 632 01767 8, Blackwell Scientific Publications, Oxford). All chemical names are run together except those of acids, acetals, esters, ethers, glycosides, ketones and salts, which are printed as separate words; hyphens are used to separate numbers, Greek letters and some configurational prefixes, e.g. *p*-nitrophenol. Italics are used for certain prefixes, e.g. *cis*-, *trans*- and *N*. Small capitals are used for dextro- and laevo-prefixes, e.g. L
Drugs. Spelling of drug names should conform with that given in the latest edition of the British National Formulary. Chemical or generic names of drugs should be used; trade names may be referred to once only upon first use of the generic or chemical

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Examples

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Sanschagrin F, Levesque RC. A specific peptide inhibitor of the class B metallo-B-lactamase L-1 from *Stenotrophomonas maltophilia* identified using phage display. *J Antimicrob Chemother* 2005; **55**: 252-5.
Journal reference (> three authors)
Williams I, Gabriel G, Cohen H et al. Zidovudine-the first year of experience. *J Infect* 1989; **18** Suppl 1: 23-31.
Whole book
Long HC, Blatt MA, Higgins MC *et al*. *Medical Decision Making*. Boston: Butterworth-Heinemann, 1997.
Book chapter
Manners T, Jones R, Riley M. Relationship of overweight to haitus hernia and reflux oesophagitis. In: Newman W, ed. *The Obesity Conundrum*. Amsterdam: Elsevier Science, 1997; 352-74.
NCCLS methods
National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically-Second Edition: Approved Standard M7-A2*. NCCLS, Villanova, PA, USA, 1990.
Meeting abstract
Hou Y, Qiu Y, Vo NH et al. 23-O derivatives of OMT: highly active against *H. influenzae*. In: *Abstracts of the Forty-third Interscience Conference on Antimicrobial*

Agents and Chemotherapy, Chicago, IL, 2003. Abstract F-1187, p. 242. American Society for Microbiology, Washington, DC, USA.

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NORM/NORM-VET 2000. Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway.
http://www.vetinst.no/Arkiv/Zoonosesenteret/NORM_VET_2000.pdf (3 May 2005, date last accessed).

For online-only journals the following reference format should be used:

Health Protection Agency. The third year of regional and national analyses of the Department of Health's mandatory MRSA surveillance scheme in England: April 2001-March 2004. CDR Wkly 2004; 14(29).
http://www.hpa.org.uk/cdr/archives/2004/cdr2904.pdf (28 June 2005, date last accessed).

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