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Evolution and Dissemination of Extended-Spectrum ß-Lactamase-Producing *K. pneumoniae*: Epidemiology and Molecular Report from the SENTRY Antimicrobial Surveillance Program During 1997-2003

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: During 2001, an epidemic of extended-spectrum ß-lactamase-producing *K. pneumoniae* (ESBL+KPN) isolates was detected in the SENTRY Program which became endemic in long-term acute care areas and the ICU in 2003. Clonal dissemination was confirmed. Similar strains were recognized from two different eastern USA cities.

Methods: Between 2001 - 2003, 123 patients in an Ohio (OH) hospital were infected or colonized with ESBL+KPN. In 1997, 1998 and 2000, 9 ESBL+KPN strains from 2 New York (NY) hospitals shared the same features as OH strains. Resistance (R) profiles were determined by reference broth microdilution methods and ribotyping was performed on all isolates. PFGE (>3 band difference = unrelated) further discriminated strains. IEF, PCR and gene sequencing were used to characterize the enzymes.

Results: The ESBL+KPN isolates from OH and NY were R to aztreonam, ceftazidime, aminoglycosides, trimethoprim/sulfamethoxazole and susceptible to ciprofloxacin and tetracycline. Molecular typing showed a single ribotype (204.2). PFGE patterns divided OH isolates into 2 subtypes (A and A_1) and the three NY strains were similar to OH isolates (A_2, A_3 and A_4). IEF of 1 NY isolate (A_4) revealed pIs at 5.4, 7.7 and 8.2. PCR and sequencing results from 1 strain of each OH and 1 NY PFGE pattern determined that TEM-1 and SHV-5 (ESBL) were present in all strains. In addition, 2 NY isolates from 1998 (A_3 and A_4) also had OXA-2. All 5 strains were negative for CTX-M series enzymes.

Conclusions: ESBL+KPN isolates with ribotype 204.2 from SENTRY Program sites have been only recognized in NY since 1997 and in OH beginning in 2001. The similarities of the antibiogram and PFGE patterns suggest that these isolates have persisted over time and may have evolved into different, but genetically related endemic ESBL+KPN clones which have the ability to cause large sustained epidemic outbreaks in the USA.

INTRODUCTION

Extended spectrum ß-lactamase (ESBL)- producing bacteria pose identification and therapeutic challenges to clinicians, infection control practitioners and clinical microbiologists. ESBLs are generally derived from TEM-1, TEM-2, and SHV-1 ß-lactamases by one or more base pair changes. These enzymes are plasmid mediated and confer resistance to broad-spectrum ß-lactams, including third- and fourth-generation cephalosporins, monobactams, and extended spectrum penicillins. Resistance to aminoglycosides and trimethoprim-sulfamethoxazole is often co-transferred on the same plasmid.

The first known ESBL-producing organism was a *Klebsiella pneumoniae* (KPN) isolate from a patient in Germany in 1983. In the last two decades, many isolations as well as large epidemic outbreaks of ESBL-producing bacteria have been reported from around the world and in the United States. ESBL production is usually observed in KPN and *Escherichia coli* and to a lesser extent in other Enterobacteriaceae or rarely in pseudomonads.

Starting in the fall of 2001, the microbiology laboratory at a midwestern hospital detected an increase in the number of resistant KPN isolates, mainly from respiratory specimens from patients located in the hospital's long-term care unit (LTAC) and ICU. The largest number of case isolates was observed in the spring of 2002 (15 occurrences). Initial DNA fingerprinting studies (reported earlier; Figure 1) indicated that the majority of isolates belonged to two related clones (A and B). A search of computer records indicated that the resistant phenotype first appeared in a LTAC patient in early August 2001. From there the organism appears to have spread to the ICU and ICU step down unit. Over 100 patients have been identified as having at least one culture positive for an ESBL-producing KPN (many were colonization). This report outlines the further epidemiological studies that were undertaken, results of molecular testing and characterization of the ESBL enzyme.

MATERIALS AND METHODS

Organism Identification: Over one hundred strains from approximately 150 patient isolates were available for further analysis. KPN isolates were initially identified in the hospital's clinical microbiology laboratory using standard commercial identification systems and confirmed at The JONES Group/JMI Laboratories (North Liberty, Iowa).

Susceptibility Testing: Initial susceptibility testing was performed by either disk diffusion or broth microdilution using a commercially prepared panel (Microscan). Screening for ESBL production was performed using the current NCCLS guidelines for zone diameters or MICs of several recommended indicator drugs. Confirmatory testing was performed using ceftriaxone and ceftazidime disks, with and without clavulanic acid.

MATERIALS AND METHODS (Continued)

Molecular Epidemiology: In-house pulsed-field gel electrophoresis (PFGE) of *Spe*1- digests was performed using the GenePath system (BioRad, Hercules, CA). Analysis of fingerprint patterns was performed by visual inspection. KPN isolates showing identical DNA banding patterns were considered to be clonal. Isolates that differed by one to three bands were considered possibly related while isolates showing > three band differences were thought to be un-related. These studies were done at two facilities, each confirming the other's results.

Epidemiologic tracking and molecular analysis was also employed using automated ribotyping (RiboPrinter™ Microbial Characterization System, Qualicon, Wilmington, DE) and discriminated using CHEF-DRII pulsed field gel electrophoresis (PFGE; BioRad Laboratories).

ß-Lactamase Typing: Plasmid extraction was carried out with QIAprep Spin Mini prep kit (Quiagen, West Sussex, United Kingdom). Transformation was performed as previously described in *E. coli* DH5α and selected in nutrient agar plates with 6 μg/ml of ceftazidime. *bla* genes were amplified using the following oligonucleotide primers: bla_{TEM} (5' ATAAAATTCTTGAAGACGAAA 3', 5' GACAGTTACCAATGCTTAATCA 3'), bla_{SHV} (5' TCGGGCCGCGTAGGCATGAT 3', 5' AGCAGGGCGACAATCCCGCG 3'), and bla_{CTX-M} (5' TTAATGATGACTCAGAGCATTC 3', 5' GATACCTCGCTCCATTTATTG 3'). PCR fragments were sequenced in both strands using DuPont Automated systems. The nucleotide and deduced aminoacid sequences were analyzed using Lasergene software package (DNASTAR, Madison, WI) and compared to sequences available over the internet.

Data Analysis: Statistical data was collected by review of patient medical charts, discharge summaries, pharmacy records, and microbiology laboratory reports.

RESULTS

- Monthly isolation of ESBL-producing KPN (ESBL+KPN) between August 2001 and February 2003 was observed. One hundred and twenty-three patients with ESBL+KPN were identified during this period. Patient age ranged from 16 to 101 with a mean of 63 yrs. Females (n=65) slightly outnumbered males (n=58).
- The majority of isolates (n=83) were from the respiratory tract (tracheal asp = 70, sputum = 4, and bronchial = 9) followed by the urinary tract (n=22). In total, 94/123 patients (76%) stayed in either the LTAC or ICU areas during their hospitalization and 21 (17%) had been in both units.
- An attempt was made to determine the clinical significance of as many ESBL+KPN isolates as possible. Of the 83 patients, 50 had information on which a clinical determination could be based. Thirty-four of 50 (68%) were believed to be infectious based on discharge summaries.
- PFGE analysis (95 patient isolates) indicated that almost all of the early isolates were clonal, pattern A (Figure 1). Some isolates recovered after April 2002, demonstrated a second but related clone pattern, designated as B.
- Since November 2002, the isolation rate of ESBL+KPN increased again and remained elevated with pattern A predominating once again. PFGE patterns showed that 64% demonstrated the A pattern, 28% demonstrated the related B pattern, and the remaining 8% showed related patterns.
- Antibiograms of 40 available ESBL+KPN (30 strains of Pattern A; 10 strains of Pattern B) are shown in Table 1. No significant differences in the dominant ß-lactam resistances were noted between the two PFGE groups. Variable susceptibility patterns were noted for some fluoroquinolones, ß-lactamase inhibitor combinations and tetracyclines. Carbapenems and polymyxins remained active.
- Gene sequencing of bla_{SHV} PCR-positive DNA was performed with the detection of TEM-1 and SHV-5 ß-lactamases. The ESBL phenotype was attributed to the SHV-5. CTX-M PCR screen tests were negative.
- The SENTRY Program data bank (1997 - date) was searched for ESBL+KPN strains having a ribotype 204.2. Only three matches were made for isolates from a hospital in New York (NY). PFGE of these strains produced similar (not identical) patterns when compared to the Ohio patterns A and B (Figure 1), subsequently retyped and interpreted as A, A_1, A_2, A_3, and A_4. The NY strains also had TEM-1 and SHV-5 and two NY isolates also contained an OXA-2 enzyme. Observed IEF results were pIs of 5.4, 7.7 and 8.2.

RESULTS

Table 1. Antimicrobial activity of 11 selected drugs tested against 40 *K. pneumoniae* strains having PFGE patterns A or B.

PFGE pattern/Antimicrobial agent	MIC (μg/ml) 50%	90%	Range	% susceptible[a]
Pattern A[b]				
Aztreonam	>16	>16	16->16	0
Ceftazidime	>16	>16	16->16	0
Ceftriaxone	16	>32	2->32	33
Gentamicin	>8	>8	>8	0
Tobramycin	>16	>16	>16	0
Amikacin	>32	>32	16->32	3
Trimethoprim/Sulfamethoxazole	>2	>2	>2	0
Ciprofloxacin	1	2	0.25->4	80
Tetracycline	≤4	8	≤4->8	63
Piperacillin/Tazobactam	16	>64	4->64	53
Imipenem	≤0.06	0.12	≤0.06-0.25	100
Pattern B[b]				
Aztreonam	>16	>16	>16	0
Ceftazidime	>16	>16	>16	0
Ceftriaxone	>32	>32	4->32	40
Gentamicin	>8	>8	>8	0
Tobramycin	>8	>8	>8	0
Amikacin	>32	>32	>32	0
Trimethoprim/Sulfamethoxazole	>2	>2	>2	0
Ciprofloxacin	1	1	0.5-4	90
Tetracycline	≤4	8	≤1-8	100
Piperacillin/Tazobactam	>64	>64	4->64	40
Imipenem	≤0.06	0.12	≤0.06-0.12	100

a. Interpretations of MIC results by NCCLS M100-S13 [2003], where available.
b. See Figure 1.

Figure 1: PFGE comparing epidemic strains A and B (lane 2 and 1, respectively; strains 004-1777 and 1778C) from the index hospital to three strains from New York (lanes 3-5; strains 015-390, 660 and 661C) sharing the same ribotype and ESBL phenotype. All strains would be in the same PFGE pattern (with subtypes) if a > 3 band criteria were applied.

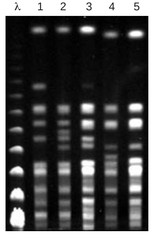

Table 2. Location of the SHV gene mutation responsible for the ESBL (SHV-5) infection-related infections/colonizations in Ohio.

ß-lactamase	Amino acid[a] at position: 39	205	238	240
TEM-1[b]	Q	Q	G	E
SHV-1	Q	R	G	E
SHV-2			S	
SHV-3		L	S	
SHV-4		L	S	K
SHV-5[b]			S	K

a. E = glutamic acid; G = glycine; K = lysine; L = leucine; Q = glutamine; R = arginine; and S = serine.
b. ß-lactamases detected in the epidemic strains (PFGE patterns A and B).

CONCLUSIONS

- The emergence and spread of ESBL+KPN as well as other Enterobacteriaceae creates a dilemma for clinicians because of the multiple drug resistance expressed by these organisms (Table 1). The true prevalence of ESBL producers is probably underestimated although better laboratory screening and confirmatory testing methodologies are now available via guidelines from the NCCLS and available commercial products/systems.
- Molecular-based epidemiologic studies of previous outbreaks have shown that the mechanisms of ESBL spread includes 1) clonal strain dissemination, and 2) clonal plasmid dissemination and selection among epidemic strains. In the present outbreak, clonal strain dissemination is responsible for the spread. A single genetic mutation, however, occurred after six months giving rise to a different but highly related DNA fingerprint (pattern B) that demonstrated slight isolation predominance (B=27, A=17,other=5) during the next seven-month period (4/02 to 11/02). Beginning in late 2002, all subsequent ESBL+KPN isolates demonstrated the A pattern. These PFGE patterns were similar to SHV-5 containing strains in NY and having the same ribotype (204.2).
- Education and intensified infection control precautions such as the use of barrier protection (gloves and gowns) and hand washing have had little affect on the continued occurrence of this organism. Additional interventions have now been employed to minimize the occurrence of the SHV-5 ESBL+KPN.

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