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The JONES Group/JMI Laboratories
North Liberty, IA, USA; www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

Clonal Dissemination and Expansion of Extended Spectrum ß-Lactamase (ESBL)-Producing *K. pneumoniae* in a Large Metropolitan Teaching Hospital: An Epidemiologic History from the SENTRY Antimicrobial Surveillance Program

J. R. DiPersio, C. Stephens, T. R. Pasquale, R. N. Jones.
Summa Health System, Akron, OH; and The JONES Group/JMI Laboratories, North Liberty, IA [www.jmilabs.com]

SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: ESBL enzymes produced by various Enterobacteriaceae seriously limit treatment options among newer ß-lactams and other antimicrobial classes due to cross-resistance (R). This epidemiologic report chronicles the initial appearance and dissemination of ESBL-producing *K. pneumoniae* (KPN) in a single hospital from 2001 into 2003.
Methods: A total of 123 cases were identified (6/01- 2/03) with each KPN isolate meeting the ESBL criteria of the NCCLS. Selected isolates were forwarded for reference MIC tests (45 agents), automated ribotyping and additional PFGE epidemiologic studies. Local surveillance and interventions were initiated. The principal ESBL phenotype was R to: aztreonam, ceftazidime, ceftriaxone, amikacin, gentamicin, tobramycin, nalidixic acid, nitrofurantoin and trimethoprim/sulfamethoxazole.
Results: Demographics of the cases were: patient age, 16-101 years (ave. 63 years), and sites of infection (respiratory tract=83, UTI=22, SSTI=11, blood=5, other=2). Index case appeared in August 2001 with highest monthly occurrence in May 2002 (15 cases). The most frequent hospital ward locations were: long-term care (59), ICU (53), or an ICU "step-down" unit (41). Molecular typing revealed a single ribotype associated with 2 dominant PFGE patterns (3 band difference; 204-2/A and 204-2/B). Pattern A predominated throughout the epidemic (August 2001 to date). Pattern B appeared between May-Oct 2002. Antibiogram subgroups (5 for A; 3 for B) were noted, each unique by R to fluoroquinolones or tetracycline, enzyme inhibitor resistance or suspected outer membrane porin changes. Carbapenems and cefepime generally remained active in vitro.
Conclusions: This epidemiologic history illustrates the danger of rapid dissemination of ESBL-producing KPN. Testing results showed: 1) epidemic clonal spread and genetic expansion; 2) target patient populations in the ICU and extended care settings; 3) variable susceptibility patterns requiring local testing; and 4) diminished risk with interventions followed by resurgence. Laboratories should be vigilant for ESBL-positive index cases.

INTRODUCTION

Extended spectrum beta-lactamase (ESBL)- producing bacteria pose increasing challenges to clinicians, infection control practitioners and clinical microbiologists. ESBLs are generally derived from TEM-1, TEM-2, and SHV-1 beta-lactamases by one or more single base pair changes. These enzymes are plasmid mediated and confer resistance to broad-spectrum beta-lactams, including third and fourth generation cephalosporins, aztreonam, 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. Since that time, many isolations as well as large outbreaks of ESBL- producing bacteria have been reported from around the world and in the United States. ESBL production is usually seen in *K. pneumoniae* and *Escherichia coli* and to a lesser extent in other Enterobacteriaceae or pseudomonads.

Starting in October 2001, the microbiology laboratory at Akron City Hospital detected an increase in the number of very resistant KPN isolates; mainly from respiratory specimens on patients located in the hospital's long-term care unit (LTAC) and ICU. The largest number of case isolates was seen in May 2002 (no.=15). Initial DNA fingerprinting studies indicated the majority of isolates belonged to two related clones. A search of computer records indicated that the resistant phenotype first appeared in a LTAC patient in early August 2001. From there the organism spread first to the ICU and then to an ICU step down unit. As of 3/1/2003, 123 patients were identified as having at least one culture positive for an ESBL-producing KPN. This report outlines the epidemiological study that was undertaken and the results of molecular testing.

MATERIALS & METHODS

Organism Identification: Nearly one hundred case isolates from 123 patients were available for further analysis. *Klebsiella pneumoniae* (KPN) isolates were initially identified in the clinical microbiology laboratory using standard commercial identification systems.

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 cefotaxime and ceftazidime disks, with and without clavulanic acid.

Molecular Epidemiology: In-house pulsed-field gel electrophoresis (PFGE) of Spe1- 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.

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)

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

RESULTS

- Figure 1 shows the monthly isolation of ESBL-producing KPN (ESBL+ KPN) between August 2001 and Feb 2003. 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). Isolates also came from wounds (n=11), blood (n=5) and cath tips (n=2). Fifty-nine patients had been in the hospitals LTAC unit during their stay. Forty of these were in the unit at the time of their first ESBL+ KPN culture. Fifty-three patients had been in the ICU for part of their stay and 28 of these were in the unit at the time of their first positive culture. 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 five patients with positive blood cultures, four were considered to be clinically significant. It was more difficult to determine the significance of respiratory isolates. Of the 83 patients, 50 had information on which a clinical determination could be based. Thirty-four of fifty (68%) were believed infected based on discharge summaries.

Table 1. Antimicrobial activity of 14 selected drugs tested against 30 *K. pneumoniae* strains having PFGE pattern A.

	MIC (µg/ml)			% by category:[a]	
Antimicrobial agent	50%	90%	Range	Susceptible	Resistant
Aztreonam	>16	>16	16->16	0	100
Ceftazidime	>16	>16	16->16	0	97
Ceftriaxone	16	>32	2->32	33	50
Gentamicin	>8	>8	>8	0	100
Tobramycin	>16	>16	>16	0	100
Amikacin	>32	>32	16->32	3	77
Trimethoprim/Sulfamethoxazole	>2	>2	>2	0	100
Nalidixic Acid	>32	>32	>32	0	100
Ciprofloxacin	1	2	0.25->4	80	10
Polymyxin B	≤1	2	≤1-2	-	3
Tetracycline	≤4	8	≤4->8	63	3
Amoxicillin/Clavulanate	8	16	8->16	67	3
Piperacillin/Tazobactam	16	>64	4->64	53	40
Ticarcillin/Clavulanate	128	>128	16->128	3	53
Imipenem	≤0.06	0.12	≤0.06-0.25	100	0
Meropenem	≤0.06	≤0.06	≤0.06	100	0

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

Table 2. Antimicrobial activity of 14 selected drugs tested against 10 *K. pneumoniae* strains having PFGE pattern B.

	MIC (µg/ml)			% by category:[a]	
Antimicrobial agent	50%	90%	Range	Susceptible	Resistant
Aztreonam	>16	>16	>16	0	100
Ceftazidime	>16	>16	>16	0	100
Ceftriaxone	>32	>32	4->32	40	60
Gentamicin	>8	>8	>8	0	100
Tobramycin	>8	>8	>8	0	100
Amikacin	>32	>32	>32	0	100
Trimethoprim/Sulfamethoxazole	>2	>2	>2	0	100
Nalidixic Acid	>32	>32	>32	0	100
Ciprofloxacin	1	1	0.5-4	90	10
Polymyxin B	2	2	≤1-2	-	0
Tetracycline	≤4	8	≤1-8	100	0
Amoxicillin/Clavulanate	8	16	8-16	50	0
Piperacillin/Tazobactam	>64	>64	4->64	40	60
Ticarcillin/Clavulanate	128	>128	32->128	0	60
Imipenem	≤0.06	0.12	≤0.06-0.12	100	0
Meropenem	≤0.06	≤0.06	≤0.06	100	0

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

- Ninety-five patient isolates collected between 8/01 to 2/03 were available for further study. PFGE analysis indicated that almost all of the early isolates were clonal and belonged to a fingerprint pattern designated as clone A (Figure 2). Some isolates recovered after 4/02, demonstrated a second but related clone pattern, designated as B. This pattern predominated through 8/02. Following epidemiologic intervention, it was thought that the nosocomial spread of this organism was minimized by 9/02 since no isolations occurred within that month and only one occurred in 10/02.

- Starting in November 2002 the isolation rate ESBL+ KPN increased again and remained elevated through February 2003 with pattern A predominating once again. PFGE patterns of the 95 patient isolates showed that 61 (64%) demonstrated the A pattern, 27(28%) demonstrated the related B pattern, and the remaining 7(7%) demonstrated patterns not related to either the A or B fingerprint.

- Antibiograms of 40 available ESBL + KPN (30 strains of Pattern A; 10 strains of Pattern B) are shown in Tables 1 and 2. No significant differences in the dominant resistances were noted between the two PFGE groups. The consistant resistances for each PFGE group were: aztreonam, ceftazidime, ceftriaxone, aminoglycosides (three drugs), trimethoprim/sulfamethoxazole, and nalidixic acid. Variable susceptibility patterns were noted for fluoroquinolones, ß-lactamase inhibitor combinations and tetracyclines. Carbapenems and polymyxins remained active, and the penicillanic sulphones were less potent among the enzyme inhibitors.

Figure 1: Epidemic strain distribution graph for ESBL-producing *K. pneumoniae* isolated by month from August 2001 through February 2003.

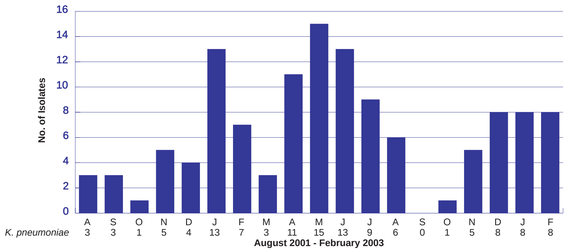
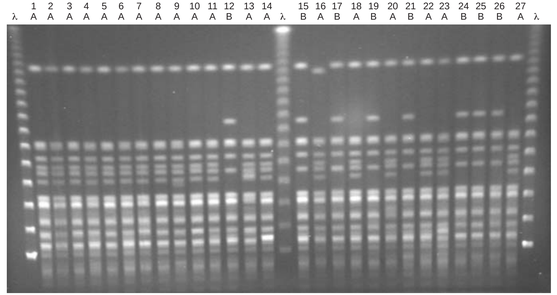


Figure 2: PFGE patterns (A and B; see header) for 27 ESBL-producing *K. pneumoniae* isolated in a single medical center in 2001-2002. λ = 48.7 kb molecular weight ladder.



CONCLUSIONS

- The emergence and spread of ESBL-producing KPN as well as other Enterobacteriaceae creates a dilemma for clinicians because of the multiple drug resistance expressed by these organisms. The true prevalence of ESBL producers is probably underestimated although better laboratory screening and confirmatory testing methodologies are now available.

- 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, however, all subsequent ESBL+ KPN isolates demonstrated the A pattern.

- Education and intensified infection control precautions such as the use of barrier protection (gloves and gowns) and hand washing have not affected the continued spread of this organism. It was initially believed that these methods were effective (see Figure 1). However, from 11/02 to 2/03 new isolations have occurred. Additional interventions have now been employed.

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