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Surveillance in Taiwan Using Molecular Epidemiology for Extended-Spectrum ß-Lactamase (ESBL)-Producing *K. pneumoniae* (KPN)

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ABSTRACT

Background: The emergence of ESBL-producing KPN has markedly reduced therapeutic choices among ß-lactams. In Taiwan, several studies have detected ESBL phenotypes, but molecular epidemiology has not been resolved.

Methods: 211 confirmed ESBL producing KPN isolates from 24 Taiwan centers were analysed by antibiogram (AB; 44 compounds), automated ribotyping (RT), PFGE and isoelectric focusing (IEF). MICs were generated by reference NCCLS methods, supported by Etest ± clavulanate to establish inhibition profiles.

Results: An AB (only 11 drugs) could achieve categorization of 53 distinct phenotypes. Excluding 12 isolates with unique ABs, RT identified 66 distinct groups, of which 39 were also unique. 11 groups were related to inter-hospital spread and 16 were due to intra-hospital dissemination. The combination of RT/PFGE identified two large epidemic clones designated 691.5/G and 595.7/A, that were from hospitals mainly in north and central Taiwan. However, major variation of the AB and IEF profiles were apparent within each clone. Isolates with the same IEF profile (pIs 7.9, 8.2, 8.4) and AB (resistance [R] to 9 compounds) were present among different molecular-typed clones, indicating spread of multi-R genomes in addition to transmission of major epidemic strains. The ESBLs (pI 7.9 or 8.4) appear to hydrolyze ceftriaxone more efficiently than ceftazidime, consistent with the CTX-M enzyme series (previously observed in Taiwan *E. coli*).

Conclusion: Rapid RT and PFGE are effective tools for large-scale analysis of genomic DNA and results can clearly identify intra- and inter- hospital dissemination in this ESBL national surveillance. The combination of phenotypic and molecular methods has proved very useful in characterizing the ESBL epidemiology of KPN in Taiwan and monitoring the risk of further spread of outbreak strains or R elements.

INTRODUCTION

Klebsiella pneumoniae (KPN) strains producing various types of extended-spectrum ß-lactamases (ESBL) enzymes have spread and increased worldwide. The frequency of KPN with ESBL-related resistance (R) has also progressively increased to a level of 10 to 30 % in Taiwan. Colonization of ESBL-producing KPN strains may involve dissemination of the epidemic stains and/or plasmid spread. In Taiwan, several studies have detected ESBL phenotypes, but nationwide molecular epidemiology has not been systematically resolved.

METHODS

MICs were generated by reference broth microdilution methods as defined by the National Committee for Clinical Laboratory standards (NCCLS) methods, supported by Etest of cefotaxime (CTX) and /or ceftazidime (CAZ) ± clavulanate to establish inhibition profiles. A total of 211 confirmed ESBL producing KPN isolates were collected from 24 hospitals in Taiwan: in northern area, 11; in central area, 5; in eastern area, 4; and in southern area, 4, from July 1998 through June 2000. These organisms were analysed by antibiogram (44 compounds), automated ribotyping (RT), PFGE and isoelectric focusing (IEF) tests.

Fig. 1. PFGE –G type of ESBL-producing KPN isolates within Ribogroup-691.5

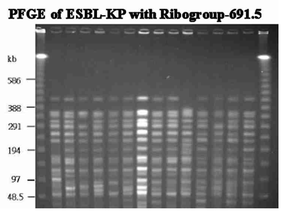
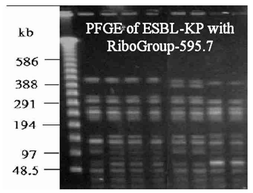


Fig. 2. PFGE-A type of ESBL-producing KPN isolates within Ribogroup-595.7



RESULTS

- Generally, resistance to ceftriaxone or cefotaxime was more prominent than cettazidime-R.
- For all ESBL-producing isolates, the carbapenems (meropenem, imipenem) were the most active (100% susceptibility [S]) followed by newer fluoroquinolones (FQs), gemifloxacin, gatifloxacin, at ~80% susceptible (S). The S rate for piperacillin-tazobactam was 79%, comparable to cefoxitin (77%), ciprofloxacin (77%) and greater than cefepime (53%).
- Co-resistance to aminoglycosides was common, gentamicin 96% R, tobramycin 96% R and amikacin 62% R.
- The use of an antibiogram (AB) with only 11 drugs could achieve epidemiologic categorization of 53 distinct phenotypes.
- Excluding 12 isolates with unique ABs, ribotyping (RT) identified 66 distinct groups, of which 39 were also unique.
- 11 groups were related to inter-hospital spread (Table 1) and 16 were due to intra-hospital dissemination (Table 2).
- The combination of RT/PFGE identified two large epidemic clones designated 691.5/G (Fig. 1) and 595.7/A (Fig. 2), that were from hospitals mainly in north and central Taiwan, respectively. However, major variation of the AB and IEF profiles were apparent within each clone (Table 3).
- Isolates with the same IEF profile (pIs 7.9, 8.2, 8.4) and AB (resistance to 9 compounds) were present among different molecular-typed clones (Table 4), indicating spread of multi-R genomes in addition to transmission of major epidemic strains.
- The ESBLs (pI 7.9 or 8.4) appear to hydrolyze ceftriaxone more efficiently than ceftazidime (Table 5), consistent with the CTX-M enzyme series (previously observed in Taiwan *E. coli*).

Table 1. Eleven RiboGroups among 115 isolates were related to interhospital spreads.

RiboGroup	No. of isolates	PFGE types and subtypes[a]	No. of involved hospitals
691.5	37	$G_{1\text{-}8}$, $H_{1\text{-}4}$	8
595.7	31	$A_{1\text{-}7}$	2
255.3	15	D, $E_{1\text{-}9}$, **1**, **2**, **3**, **4**	9
73.2	10	$B_{1\text{-}5}$	3
746.6	4	$F_{1\text{-}3}$	3
98.4	4	C	2
80.8	4	$I_{1\text{-}2}$	2
96.3	3	$J_{1\text{-}2}$	2
88.2	3	$K_{1\text{-}3}$	2
92.1	2	$K_{1\text{-}5}$	2
109.3	2	$L_{1\text{-}2}$	2

[a]The alphabet represents the designation of PFGE type with epidemic clone. The subscript number after the alphabet represents the PFGE subtype, whereas the boldface number represents the unique PFGE type different to any alphabetical PFGE type.

Table 2. Isolated intrahospital dissemination occurred among 42 isolates.

Hospitals	RiboGroup (number of isolates)	PFGE (no. of types)	Isolates no.
A	73.8 (3); 75.1 (2); 203.3 (2); 1012.5 (2)	4	9
B	79.3 (4); 87.8 (4); 615.2 (4); 75.8 (3); 78.7 (3); 79.2 (3); 76.1 (2); 76.2 (2); 77.4 (2)	9	27
C	94.8 (2); 95.5 (2)	2	4
D	109.6 (2)	1	2

CONCLUSIONS

- We found that 83 (39%) isolates had a CTX-M phenotype characteristic of high MICs for ceftriaxone and aztreonam but low MIC level for ceftazidime. As we know, CTX-M-type ß-lactamases have not been reported among klebsiellae isolates in Taiwan. Among those isolates with CTX-M phenotype, 50 were randomly selected for IEF analysis, which revealed that 22 contained pI of 7.9 and 28 contained pI of 8.4 (Table 5).
- The ß-lactamases of pI 5.4 and 7.6 have been reported to be TEM-1 and SHV-1, respectively, in Taiwan hospitals.
- Similar to previous reports, SHV-5-type ß-lactamase (pI 8.2) has been regarded as the most common type of ESBL among KPN strains in Taiwan.
- The surveillance study identified 11 clusters of interhospital clonal dissemination, of which, two large clusters of RT-691.5/G and RT-595.7/A were isolated from the hospitals mainly in the Northern and Middle area of the country.
- The present study was most consistent to reports published by Yuan *et al* who documented wide diversity in antibiogram, plasmid pattern, and ß-lactamase profile within some epidemic clones, indicating a dynamic situation of plasmid genomes in ESBL-producing KPN strains.
- Rapid RT and PFGE were very effective tools for large-scale analysis of genomic DNA and results clearly identify intra- and inter- hospital dissemination in this ESBL national surveillance.
- The combination of phenotypic and molecular methods has proved very useful in characterizing the ESBL epidemiology of KPN in Taiwan and monitoring the risk of further spread of outbreak strains or R elements. Nation-wide monitoring coupled with efforts to contain the spread of these isolates is an important priority.

Table 3. The antibiogram and IEF profiles of RT-691.5/G and RT-595.7/A clones.

Clones (RT/PFGE)	Antibiogram[a]	IEF profiles
691.5/G	1a.2, 1a.3, 2a.2, 3a.1, 3a.2, 3d.2, 4d.2, 5a.2, 5d.2	(7.6, 8.2); (5.4, 7.6, 8.2); (5.4, 7.6, 8.2, 8.8); (5.4, 7.6, 7.9, 8.2, 8.4)
595.7/A	1a.1, 1b.1, 1c.1, 1d.1, 1e.1, 1c.3, 2b.1, 2c.1, 2e.1	(5.4, 7.9); (5.4, 8.4); (5.4, 7.6, 7.9); (5.4, 7.6, 8.2); (5.4, 7.6, 8.4); (5.4, 7.9, 8.2); (5.4, 7.6, 7.9, 8.2); (5.4, 7.6, 7.9, 8.4); (5.4, 7.0, 7.6, 7.9, 8.2)

[a]the antibiogram was designated x or y or z according to MIC (µg/mL) as followings:

x	TET	TMX	GM	TOB	AN	y	CRO	CFP	CAZ	ATM	z	FOX	CIP
1	R	R	R	R	R	**a**	>8	>8	>8	>8	**1**	≤8	≤1
2	S	R	R	R	R	**b**	>8	>8	≤8	>8	**2**	>8	≤1
3	S	R	R	R	S	**c**	>8	≤8	≤8	>8	**3**	>8	>1
4	S	R	S	R	S	**d**	>8	≤8	>8	>8	**4**	≤8	>1
5	R	R	R	R	S	**e**	>8	≤8	≤8	≤8			

Abbreviation: TET: tetracycline; TMX: trimethoprim/sulfamethoxazole; GM: gentamicin; TOB: tobramycin; AN:amikacin; CRO: ceftriaxone; CFP: cefepime; CAZ: ceftazidime; ATM: aztreonam; FOX: cefoxitin; CIP: ciprofloxacin; S: susceptible; R: non-susceptible (NCCLS MIC breakpoint)

Table 4. Different clones of isolates with the same IEF profile and antibiogram (1a type).

RiboGroup	PFGE	Antibiogram	Isolate no.	ESBL pIs[a]
691.5	G_1, H_1	1a.3	2	7.9, 8.2, 8.4
691.5	G_2	1a.2	1	7.9, 8.2, 8.4
73.2	B_1	1a.1	2	7.9, 8.2, 8.4
80.8	I_2	1a.1	1	7.9, 8.2, 8.4
746.6	F_2	1a.3	1	7.9, 8.2, 8.4

[a]The enzymes with pIs of 5.4 and/or 7.6 may be contained.

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Table 5. Distribution of MICs (µg/mL) of different ß-lactams against KPN isolates with CTX-M-type enzymes

Antibiotics	N of isolates at MIC (µg/ml):								
MIC for strains with enzyme of:	≤ 0.5	1	2	4	8	16	>16	32	>32
pI 7.9[a] (N=22)									
Cefoxitin	0	0	6	12	2	0	_	0	2
Ceftriaxone	0	0	0	0	0	0	_	1	21
Ceftazidime	0	1	13	6	2	0	0	_	_
Aztreonam	0	0	0	0	3	8	11	_	_
Cefepime	0	0	0	1	5	5	11	_	_
Piperacillin-tazobactam[b]	0	0	5	5	9	2	_	0	1[c]
pI 8.4[a] (N=28)									
Cefoxitin	0	3	11	11	2	0	_	0	1
Ceftriaxone	0	0	0	0	0	1	_	2	25
Ceftazidime	4	9	6	6	3	0	0	_	_
Aztreonam	0	0	0	1	8	8	11	_	_
Cefepime	0	0	3	5	13	1	6	_	_
Piperacillin-tazobactam[b]	0	2	11	11	1	1	_	2	0

Note. pI: isoelectric point. _: not included in the scale of microdilution tray panel.
[a]All strains contained an enzyme of pI 5.4 and may plus an additional pI 7.6 enzyme.
[b]Tazobactam was at a fixed concentration of 4 µg/mL
[c]MIC = 64 µg/mL