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Persistent Occurrence of Clonally Related Multi-Drug Resistant *Acinetobacter* spp. Isolates Within the MYSTIC Programme Medical Centers (USA, 1999-2004)

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MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

ABSTRACT

Background:The Meropenem (MEM) Yearly Susceptibility Test Information Collection (MYSTIC) Programme is a longitudinal resistance (R) surveillance network of 117 medical centers worldwide created to monitor the carbapenems and other broad-spectrum antimicrobial agents. In the USA, 10 - 16 medical centers routinely participate by submitting 200 non-duplicate bacterial isolates from clinical infections with target totals for specified species or groups.

Methods: Between 1999 and 2004, a total of 489 *Acinetobacter* spp. (ASP) strains were collected by the central laboratory (JMI Laboratories) and tested for susceptibility (S) using CLSI/NCCLS reference methods and interpretative criteria for 12 broad-spectrum antimicrobial agents. Isolates within a participant hospital with multi-drug resistant (MDR) antibiograms (≥ 3 classes R) were further characterized by automated ribotyping and pulsed-field gel electrophoresis (PFGE) methods to determine possible clonality and epidemic dissemination.

Results: Only amikacin, tobramycin, imipenem and meropenem have demonstrated > 80% S against the ASP strains collected in the MYSTIC Programme for any monitored year. The most frequently observed ASP ribotype (931.7) has been observed in 49 of the 96 ASP strains tested (1999 - 2004) from five medical centers including sites in Colorado, Delaware and New York (NYC; 3 sites). This MDR ASP ribotype has persisted annually at site 04 and has occurred in 4 of the 6 years for the other institutions. The second most frequent ribotype pattern (1110.4) encompasses 8 strains, all collected in 2004 from 2 sites. A total of 10 other ribotype patterns have been detected among 31 MDR ASP strains with a range of 2 to 5 strains/pattern.

Conclusions: A single, dominant clone of MDR ASP has been identified in the MYSTIC Programme participant sites accounting for over 10% of the total ASP isolates collected. This ribotype has persisted in NYC and appears endemic in this region, although non-clonal occurrences have been noted elsewhere. Continued surveillance of Gram-negative pathogens is warranted to monitor the presence and possible spread of these problematic MDR clones.

INTRODUCTION

Non-fermentative Gram-negative bacilli including *Acinetobacter* spp. are ubiquitous organisms widely distributed in nature. Since the 1980's they have emerged as important multidrug-resistant opportunistic pathogens primarily causing nosocomially acquired infections such as pneumonia, bacteremia, meningitis, urinary tract and surgical wound infections. Surveillance study results have reported *Acinetobacter* spp. as the fourth most prevalent pathogen representing 7 - 10% of bacterial isolates from lower respiratory tract cultures from patients hospitalized with pneumonia in Latin America. *Acinetobacter* spp. is also among the 10 most frequently isolated pathogens from bloodstream infections, representing 2 - 4% of isolates depending on the geographic region monitored.

The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme is a longitudinal resistance surveillance study with 117 participant sites in Europe, North America, Latin America and Asia. The MYSTIC Programme monitors the in vitro activity of meropenem and other broad-spectrum antimicrobial agents. Medical centers have been monitored in the United States (USA) since 1999 by a central processing laboratory (JMI Laboratories, North Liberty, Iowa, USA) using reference susceptibility testing methods to detect resistance rate changes to carbapenems and comparator broad-spectrum antimicrobial agents. The purpose of this report was to summarize the susceptibility patterns and the change in resistance rates among *Acinetobacter* spp. isolates over the six year period of the MYSTIC Programme (USA; 1999 - 2004), and also to evaluate the influence of the clonal spread of multidrug-resistant (MDR) strains on current observed resistance rates.

MATERIALS AND METHODS

The MYSTIC Programme in the USA has utilized 10 - 16 medical centers geographically dispersed across the USA. The study protocol for 2004 outlined specific quotas among Gram-negative species for a total of 200 isolates per center, each isolate originating from serious infections. Due to their intrinsic, enzyme-mediated resistances to carbapenems, *Stenotrophomonas maltophilia* and *Chryseobacterium* spp. were excluded. All isolates were submitted to the central laboratory (JMI Laboratories, North Liberty, Iowa, USA) on provided transport swabs.

During 2004, a total of 2,799 Gram-negative isolates were processed and over the six year study period (1999 - 2004), 15,990 bacterial isolates were collected. Organism identifications were performed locally with identification confirmation achieved using colonial morphology, biochemical tests (Remel, Lenexa, Kansas, USA) and/or the Vitek System identification cards (bioMerieux, Hazelwood, Missouri, USA) at the monitoring laboratory as required. All isolates were stored at -70°C in trypticase soy broth with 20% glycerol, except streptococci (-70°C in defibrinated rabbit blood) and *Pseudomonas* spp. (room temperature in sterile water).

Susceptibility testing was performed for all bacterial strains utilizing Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards [NCCLS]) reference methods published in M7-A6 to determine minimum inhibitory concentrations (MICs). The antimicrobial agents tested were: meropenem, imipenem, aztreonam, cefepime, ceftazidime, ceftriaxone, ceftizoxime, piperacillin/tazobactam, amikacin, gentamicin, tobramycin, ciprofloxacin and levofloxacin. CLSI/NCCLS criteria published in M100-S15 were applied for interpretation of susceptibility and resistance. Concurrent testing with American Type Culture Collection (ATCC) strains *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 assured the quality control of the susceptibility test methods.

All isolates resistant to meropenem (≥ 16 μg/ml), imipenem (≥ 16 μg/ml) and ceftazidime (≥ 32 μg/ml) were screened for the production of a metallo-ß-lactamase enzyme using a disk approximation method to demonstrate EDTA or 2-mercaptopropionic acid inhibition of meropenem, imipenem or ceftazidime hydrolysis.

Multidrug-resistant isolates were typed for genotypic relatedness using automated ribotyping (RiboPrinter™ Microbial Characterization System, Qualicon, Wilmington, DE, USA) of genomic DNA after digestion using EcoRI enzyme. Separation of the DNA fragments by agarose gel electrophoresis resulted in banding patterns which could be captured by image analysis software and compared to all previously tested isolates to assign a ribogroup. Further discrimination of ribogroups, when necessary, was performed using CHEF-DRII pulsed-field gel electrophoresis (PFGE; BioRad Laboratories, Hercules, CA, USA) on DNA digested with SmaI restriction enzyme. Gels were stained with ethidium bromide to visually identify banding patterns and determine clonal relatedness.

RESULTS

- The susceptibility testing results for the *Acinetobacter* spp. strains collected by the MYSTIC Programme in the 1999 - 2004 period are summarized in Table 1.
- In 2004, only amikacin (84.5%), tobramycin (84.5%), imipenem (83.8%) and meropenem (76.1%) demonstrated acceptable susceptibility rates against the *Acinetobacter* spp. isolates tested.

RESULTS CONTINUED

- Most monitored antimicrobial agents demonstrated increased rates of resistance during the 1999 - 2004 period. Ceftazidime, ciprofloxacin and cefepime all showed ≥ 19.2% increase in resistance rates. Only meropenem (5.7% decrease) and tobramycin (12.3% decrease) demonstrated reductions in resistance rates from the 1999 baseline results, but each had an increase in resistance compared to the prior year.
- None of 34 MDR *Acinetobacter* spp. isolates screened (ceftazidime ≥ 32 μg/ml, imipenem ≥ 16 μg/ml, and meropenem ≥ 16 μg/ml) for the presence of metallo-ß-lactamase were positive.
- Ribotype 105.931.7 was identified for *Acinetobacter* spp. isolates from five MYSTIC Programme sites (New York [3], Delaware and Colorado). This ribogroup accounted for 49 of the 96 (51.0%) MDR strains (Table 2; Figure 1). PFGE results confirmed clonal dissemination within and between medical centers.

Table 1. Antimicrobial activity of meropenem and selected broad-spectrum comparator agents against *Acinetobacter* species isolates from the USA MYSTIC Programme (1999-2004).

	2004			1999	2000	2001	2002	2003
Organism (no. tested)/ antimicrobial agent	MIC_{50}	MIC_{90}	% S/R[a]	% S/R	% S/R	% S/R	% S/R	% S/R
Acinetobacter spp. (no. tested)[b]		(142)		(32)	(56)	(79)	(69)	(111)
Meropenem	0.5	16	76.1/16.2	78.1/21.9	78.6/19.6	81.0/19.0	84.1/13.0	87.4/7.2
Imipenem	0.25	8	83.8/8.5	81.3/6.3	80.4/10.7	83.5/11.4	88.4/11.6	91.9/1.8
Ceftriaxone	32	>32	21.1/40.8	34.4/25.0	25.0/33.9	25.3/32.9	34.8/31.9	36.0/35.1
Ceftazidime	16	>16	49.3/41.5	68.8/18.8	66.1/28.6	64.6/29.1	58.0/34.8	64.0/32.4
Cefepime	8	>16	51.4/31.7	68.8/12.5	60.7/26.8	51.9/26.6	53.6/27.5	63.1/18.0
Aztreonam	>16	>16	12.0/72.5	15.6/59.4	3.6/83.9	12.7/78.5	13.0/63.8	8.1/67.6
Piperacillin/Tazobactam	16	>128	54.9/37.3	71.9/21.9	58.9/23.2	70.9/21.5	62.3/18.8	61.3/16.2
Gentamicin	≤1	>8	63.4/33.8	65.6/34.4	64.3/33.9	62.0/31.6	59.4/30.4	63.1/32.4
Tobramycin	≤1	>8	84.5/12.7	71.9/25.0	78.6/16.1	73.4/26.6	71.0/21.7	82.0/11.7
Amikacin	≤4	>32	84.5/14.1	-	-	-	-	-
Ciprofloxacin	0.5	>2	54.2/44.4	71.9/25.0	62.5/35.7	59.5/38.0	56.5/40.6	58.6/40.5
Levofloxacin	0.25	>8	59.9/39.4	-	-	-	-	60.4/36.0

a. Criteria as published by the CLSI/NCCLS [2005].
b. Includes: *Acinetobacter baumannii* (354 strains), *A. calcoaceticus* (two strains), *A. junii* (four strains), *A. lwoffii* (45 strains) and *Acinetobacter* spp. (84 strains).

Table 2. Molecular typing results for MDR *Acinetobacter* spp. isolates tested in MYSTIC Programme 1999 - 2004 in USA.

					Year					
Organism	Site	No. strains	Ribotype	PFGE	1999	2000	2001	2002	2003	2004
A. baumannii	02	11	105.931.7	B/B1/C/C1		X	X	X		X
	04	26	105.931.7	A/A1/B/B1/C1/C2/D/D2	X	X	X	X	X	X
	06	7	105.931.7	C/D/D3		X	X			
	17	2	105.931.7	NT				X		
	18	3	105.931.7	A/B/D1				X		X
	17	3	105.1110.4	A						X
	24	7	105.1110.4	A1						X
	02	3	105.218.3	A1/A2						X
	02	2	105.1221.5	A/A1	X					
	06	4	105.1090.2	A/A1			X			
	11	5	258.192.1	A/B/B1					X	
	18	2	105.717.2	C						X
	12	1	105.531.3	NT		X				
	22	3	105.531.3	A						X
	22	5	258.243.1	A						X
	22	2	258.243.3	B						X
	24	2	105.393.6	A1						X

- The second most common ribotype observed (105.1110.4) was identified in 10 *Acinetobacter* spp. isolates, all of them isolated in 2004 from two western medical centers (Figure 1). PFGE typing demonstrated nearly identical results also indicating clonal similarities between institutions. Another ribotype (105.531.3) was common to medical centers in Georgia and Tennessee, but clonal evaluation by PFGE was not possible at one site (Georgia).
- A total of 17 additional ribogroups were identified among the remaining 37 MDR *Acinetobacter* spp. isolates (range 1 - 5 isolates/ribogroup).

Figure 1. Example of the two most commonly observed (59 strains) riboprinter patterns in the MDR *Acinetobacter* spp. isolates identified within the MYSTIC Programme (1999 - 2004).

Isolate	Label	Site	City, State	Year of Isolation	RiboGroup	RiboPrint™ Pattern (1 kbp, 5, 10, 15, 50)
1	728	18	Wilmington, DE	2002	105-931-S-7	
2	107	2	New York, NY	2000	105-931-S-7	
3	238	4	New York, NY	2002	105-931-S-7	
4	295	6	New York, NY	2000	105-931-S-7	
5	1404	17	Denver, CO	2002	105-931-S-7	
6	2502	17	Denver, CO	2004	105-1110-S-4	
7	2835	24	Seattle, WA	2004	105-1110-S-4	

CONCLUSIONS

- Annual MYSTIC Programme susceptibility testing results for *Acinetobacter* spp. isolates showed a remarkable increase in rates of resistance for many broad-spectrum agents for 2004, but the activities of the carbapenems remained acceptable during the 1999 - 2004 period.
- Evidence of clonal spread of MDR *Acinetobacter* spp. isolates both within individual medical centers and within a geographic region significantly affected the resistance rates of the antimicrobial agents monitored by the MYSTIC Programme.
- The largest clone of *Acinetobacter* spp. isolates was observed in the New York City area from three medical centers including one center having MDR isolates in all six monitored years (1999 - 2004).
- Continued surveillance of non-fermentative Gram-negative bacilli, including *Acinetobacter* spp., appears warranted to monitor for changing susceptibility trends in the activity of carbapenems and other broad-spectrum antimicrobial agents.

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