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ROLE OF CLONAL OCCURRENCES OF MULTI-DRUG-RESISTANCE IN THE MYSTIC PROGRAMME (USA; 1999-2003)

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MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

ABSTRACT

Background
The Meropenem (MEM) Yearly Susceptibility Test Information Collection (MYSTIC) Programme was initiated in 1997, but in 1999 for the USA. This program monitors resistance (R) in participant centers where carbapenems are prescribed and drug use data are obtained. An earlier report found antimicrobial use was not a clear cause of local or aggregate changes in R rates (Mutnick et al., JAC 2004). This study addresses the role of dissemination of R clones on R rates for non-fermentors *Acinetobacter* spp. (ACB) and *P. aeruginosa* (PSA).
Methods
Carbapenem (CARB)-multi-drug R (MDR) strains from among 226 ACB and 1,112 PSA were tested by reference broth microdilution methods, automated ribotyping and PFGE to determine possible clonal dissemination. Each strain was also tested for metallo-ß-lactamases (MBL) and then analyzed by CARB-R rate (phenotypic and PCR) and DDD/100 days use groupings (high, moderate, low).
Results
For the aggregate 15 sites in the MYSTIC Programme each year, the CARB-R rate decreased over 5 years; but other drug-R rates generally escalated. ESBL-R rates were stable in *E. coli* and *Klebsiella*. Changes were not related to use calculations. Discovered clonally spread strains were elevated in high-R (1.8 clones/site) and moderate-R rate centers, compared to unique MDR-PSA in low-R hospitals. ACB clonality was extreme in one geographic area with dissemination of 5 clones in 4 centers. R-rates in ACB and PSA were clearly related to clonal occurrence and spread, and one MBL (VIM-7; Toleman et al., AAC 2004) was detected representing it's persistence in a Texas site. Decreased CARB-R rates from 1999-2002 was directly attributed to disappearance of R clones in some locations.
Conclusions
ACB and PSA CARB- and MDR-R rates in MYSTIC Programme institutions have been greatly influenced by clonal dissemination, less by antimicrobial use patterns. Most severe examples of clonality were observed among ACB in New York City and the documented endemic nature of VIM-7 PSA (0.9% of all PSA isolates). MEM remained the most active agent tested in the program and surveillance networks must implement epidemiologic typing to assess the role of clonal spread on the R rates.

INTRODUCTION

The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme is a global and longitudinal surveillance network of medical centers utilizing carbapenems, especially meropenem. Institutions have been monitored internationally since 1997 (1999 in the USA), using reference susceptibility methods as described by the National Committee for Clinical Laboratory Standards (NCCLS), to detect emerging resistance patterns to carbapenems and comparator broad-spectrum antimicrobial agents. The defining aspect of this program has been the intent to capture antimicrobial consumption data from participating institutions, leading to the evaluation of usage patterns, individually or in aggregate, compared with emerging resistance patterns of carbapenems and other classes of antimicrobials.

The purpose of the study was to assess the role of clonal spread of multi-drug-resistant (MDR) strains of *Acinetobacter* spp. and *Pseudomonas aeruginosa* on the observed patterns and rates of resistance in the MYSTIC Programme (USA, 1999-2003). Over the initial three year period of the MYSTIC Programme, various patterns of resistance were identified that did not have a direct relationship to the monitored use of corresponding antimicrobials [Mutnick et al., 2004]. The use of aggregate resistance rates and use statistics (DDD/100 patient days) was misleading as outlined by further medical center specific calculations. Clearly, other confounding variables were influencing the often dramatic changes in resistance rates to some agents such as the carbapenems (decreased resistance with increased use), and ciprofloxacin (increased resistance with increased use). To address this paradox, we further examined the available *Acinetobacter* spp. and *P. aeruginosa* isolates by molecular epidemiology methods from all medical centers participating in the MYSTIC Programme from 1999 through 2002. Each strain that exhibited a resistance to a monitored carbapenem (MIC of ≥ 16 µg/ml for imipenem or meropenem) was selected for additional epidemiologic typing and enzyme mechanism testing.

MATERIALS AND METHODS

Twenty-three medical centers participated over the four monitored years (10 - 15 sites/year). The number of organisms processed and available for follow-up tests were: *Acinetobacter* spp. (226 strains) and *P. aeruginosa* (1,112 strains). Initial screens for clonality of MDR strains (resistant to carbapenems [imipenem and meropenem] and various other anti-pseudomonal agents) revealed a significant subset collection of *Acinetobacter* spp. (36 strains) and *P. aeruginosa* (118 strains).

The data analyses were further classified by the rates of carbapenem resistance which fell into three distinct groups: 1) low rates at 0 - 7% (median, 3%); 2) moderate rates at 11 - 15% (median, 13%); and 3) high rates at 18 - 31% (median at 19%). A total of 10, eight and five medical centers were found in each category, respectively. Furthermore, the carbapenem use rates were grouped into 1) low rates (< 15 DDD/100 patient days); 2) moderate rates (24 - 28 DDD/100 patient days); and 3) high rates (48 - 83 DDD/100 patient days).

MATERIALS AND METHODS CONTINUED

Epidemiologic typing of the carbapenem-resistant strains of *Acinetobacter* spp. and *P. aeruginosa* was accomplished by automated ribotyping (Qualicon Riboprinter) and by pulsed field gel electrophoresis (PFGE).

Susceptibility testing was performed by the reference broth microdilution method [NCCLS, 2003] and interpretations as published in the NCCLS M100-S14 tables [2004]. Metallo-ß-lactamase screening criteria were those established in Japan by Senda et al. [1995 and 1996] that includes resistance level MIC values to imipenem (≥ 16 µg/ml), meropenem (≥ 16 µg/ml) and ceftazidime (≥ 32 µg/ml). Metallo-ß-lactamase phenotype tests were also applied by the disk approximation test using two enzyme inhibitors (EDTA, 2-MPA) and four substrate ß-lactams (imipenem, meropenem, aztreonam, ceftazidime). Disk spacing was 20 mm (edge to edge) and an enhanced zone of inhibition between the chelator and substrate disks was defined as suspicious or presumptive evidence for a metallo-ß-lactamase-producing isolate. Confirmation of phenotype-positive isolates was performed by carbapenem hydrolysis assays (imipenem and meropenem) and PCR tests using selected primer sets for bla_{IMP}, bla_{VIM}, bla_{SPM}, as well as the OXA-series enzyme, OXA-45.

RESULTS

- Table 1 shows the trends in resistance rates for four (4) selected Gram-negative pathogens for the monitored interval of 1999 - 2003. For the two non-fermentative Gram-negative species groups, the meropenem resistance rates generally declined over the five years. In contrast, the susceptibility rates for ceftazidime remained stable against the *E. coli* and *Klebsiella* spp. isolates. Also the rates of ESBL-producing phenotypes were unchanged or decreasing in prevalence (*E. coli*) among MYSTIC Programme hospitals. Aggregate resistance trends and meropenem usage rate data revealed a significant inverse correlation as reported earlier by Mutnick et al. [2004].
- Clonal analysis for the *P. aeruginosa* isolates was divided into the three categories of endemic carbapenem resistance (low, moderate, high; Table 2). Among the centers with high endemic rates of resistance, the clonality of these MDR-*P. aeruginosa* was evident with an average of 3.6 strains of clonal origin compared to 10.6 unique isolates per center (29.5% of strains were clonal). The results were similar for the moderate carbapenem resistance group of medical centers, where five clones were identified (10 total strains). The unique MDR-*P. aeruginosa* isolates numbered 36 strains for an overall clonal proportion of 21.7%, but a lower rate of clones per center. Lastly, medical centers with low MDR-*P. aeruginosa* rates had no evidence of clonal spread.
- Also evident from the results listed in Table 2, the clonal strain isolations decreased over time to only one persisting clone (site 03) in 2002. This decline in MDR-*P. aeruginosa* clones may be the result of local infection control efficacy. This reduction in clonal occurrence appears to be responsible for the observed reduction in carbapenem resistance since the vast majority of study sites were consistent across the monitored interval.
- Table 3 summarizes the clones discovered among the MDR-*P. aeruginosa* that were noted in more than one center. Site 02 and 06 were in the same city (New York); and sites 01 and 07 were in Texas and California, respectively.
- Table 4 shows the dominant clonal patterns of MDR-*Acinetobacter* spp. isolates and Figure 1 illustrates the five ribotypes. The most frequently encountered clones were 931.7/C and 931.7/D. The clone 931.7/C was discovered in all three New York sites (02, 04, 06), and 931.7/D in New York (04, 06) and Wilmington, Delaware. Three other clones were also discovered in the New York institutions (931.7/B, 1090.2/A, 167.5/A), each unique to a participant site.
- A total of 61 strains from both non-fermentative Gram-negative species groups had elevated carbapenem and ceftazidime MIC values consistent with a metallo-ß-lactamase. Two and three strains were suspicious for harboring a metallo-enzyme after the phenotypic, disk approximation screening test for *Acinetobacters* and *P. aeruginosa*, respectively. The *Acinetobacter* spp. isolates were negative by hydrolysis tests, but one *P. aeruginosa* hydrolyzed the carbapenems and was determined to be a VIM-7 enzyme by specific PCR primer set testing.
- Table 5 shows the correlation of carbapenem use (by categories) with the endemic resistance rates for the carbapenems (meropenem). Also noted in Table 5 are the results of the clonality study (see footnotes). All nine compartments of this comparison are populated with results and no clear relationship between carbapenem use and resistance can be identified. However, clonality appears to, impact the level of carbapenem resistance.

Table 1. Activity as measured by susceptible and resistance rates for meropenem and ceftazidime tested against *Acinetobacter* spp. (347 strains), *P. aeruginosa* (1,565 strains), *E. coli* (1,608 strains) and *Klebsiella* spp. (1,161 strains) by NCCLS reference MIC methods.

	% susceptible/resistant:[a]				
Organism/antimicrobial	1999	2000	2001	2002	2003
Acinetobacter spp. (no. tested)	(32)	(56)	(79)	(69)	(111)
Meropenem	78.1/12.5	78.6/19.6	81.0/19.0	84.1/13.0	87.4/7/2
P. aeruginosa (no. tested)	(193)	(299)	(298)	(321)	(454)
Meropenem	78.2/16.1	84.3/10.0	85.9/8.4	93.1/4.4	88.3/7.3
E. coli (no. tested)	(197)	(313)	(306)	(323)	(469)
Ceftazidime	97.0/2.5	98.1/1.9	97.1/2.3	99.4/0.3	99.6/0.4
ESBL phenotypes (%)	5.1	3.2	3.3	2.8	1.1
Klebsiella spp. (no. tested)	(152)	(233)	(225)	(248)	(303)
Ceftazidime	96.1/3.3	94.4/5.2	93.8/5.3	97.6/2.4	95.4/4.0
ESBL phenotypes (%)	7.2	6.9	7.1	3.6	6.9

a. Criteria of the NCCLS [2004].

Table 2. Distribution of unique and clonal MDR-*P. aeruginosa* listed by category of carbapenem resistance rates in MYSTIC Programme (USA, 1999 - 2002).

	No. MDR strains		Year of clonal strain isolation			
Resistance category/ participant no.	Unique	Clonal (clones)	1999	2000	2001	2002
High						
01	9	3(2)	X	X	X	
04[a]	15	2(1)	X	X		
06[a]	8	11(5)[b]	X	X	X	
10	7	2(1)	X			
12	4	0				
Moderate						
02[a]	5	4(2)[c]		X	X	
03	3	2(1)	X			X
05	4	0				
07	6	1(1)[c]	X			
08	7	0				
14	5	3(1)	X			
15	4	0				
22	2	0				
Low						
09	1	0				
11	1	0				
13	0	0				
16	0	0				
17	2	0				
18	1	0				
19	0	0				
20	0	0				
21	1	0				
23	1	0				

a. Also have MDR-*Acinetobacter* clones.
b. Clone shared with site 02 among the moderate resistance group.
c. Clone shared with site 06 and 01, respectively, among the high-resistant rate hospitals.

Figure 1: MDR *Acinetobacter* clones (5) by ribotype in the MYSTIC Programme (1997-2002).

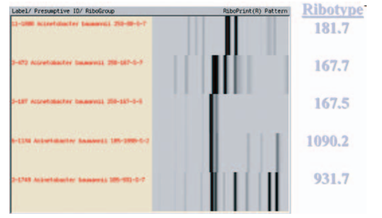

Table 3. Distribution of MDR-*P. aeruginosa* clones among MYSTIC Programme centers (USA; 1999 - 2002).

Ribotype/PFGE pattern by site (no.)[a]			
06	02	01	07
272.1/AA(1)	272.1/AA(1)	151.2/DD(2)	
566.6/O(4)	21.3/NN(2)		
191.6/A(2)		162.4/GG1(1)	162.4/GG1(1)
163.1/JJ(2)			
163.1/KK(2)			

a. Four other sites (03, 04, 10, 14) had clonal occurrences of 158.6/FF(2), 528.5/G1, 566.4/J and 780.4/Z that were unique to that medical center.

Table 4. Clonal epidemiology of MDR-*A. baumannii* (MYSTIC Programme, USA; 1999 - 2002).

	Occurrences by ribotype and PFGE pattern							
	931.7				1090.2	167.5	167.7	181.7
Site (no. tested/state)	*	B	C	D	A	A	NT	NT
02(10/NY)	-	-	7	-	-	2	1	-
04(13/NY)	1	3	5	4	-	-	-	-
06(11/NY)	-	-	4	3	4	-	-	-
18(1/DE)	-	-	-	1	-	-	-	-
11(1/LA)	-	-	-	-	-	-	-	1

a. * = undeterminable; NT = not tested.

Table 5: Correlation of qualitative categories of carbapenem resistance versus DDD/100 bed days (100d) expressed in qualitative groupings.

	Resistance (%)[a]		
Use[a] (DDD/100 d)	Low (0-7)	Moderate (11-15)	High (18-31)
High (40-82)	1[b]	1[b]	1[b]
Moderate (24-28)	1	1[b]	1[b,c]
Low (< 15)	4	4[b,c]	1[b]

a. Average DDD and resistance rates in % over 2 - 4 years.
b. Includes clonal sites for *P. aeruginosa*.
c. Includes clonal sites for *Acinetobacter* spp.

CONCLUSIONS

- Clonal emergence and spread contributed significantly to the carbapenem resistance rates among non-fermentative Gram-negative bacilli observed in the MYSTIC Programme (USA/1999 - 2003), in contrast to low correlations reported earlier for broad-spectrum antimicrobial use.
- Epidemic effects were greatest with Acinetobacters in the New York City area and less with *P. aeruginosa* where many unique, non-clonal MDR strains were detected.
- Poor correlations between carbapenem use and resistance was attributed to varying levels of endemic resistant strains isolated in each institution and the persistence of or declines of endemic/epidemic clones.
- MDR clones among non-fermentative Gram-negative bacilli have decreased over the monitored/study interval (1999 - 2003) of the MYSTIC Programme, and metallo-ß-lactamases remain extremely rare (one documented occurrence in 1,112 strains; 0.09% for VIM-7). Earlier experience with a *P. aeruginosa* strain from a participating medical center in Houston, Texas revealed the first USA-based isolation of a metallo-ß-lactamase. This strain also produced a unique OXA-45, class 2d' enzyme, not found in this clonality study. These findings are consistent with the persistence of the first metallo-ß-lactamase (bla_{VIM-7}) within the MYSTIC Programme medical center and in the USA.

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