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OCCURRENCE, PERSISTENCE AND INFLUENCE OF RESISTANT GRAM-NEGATIVE CLONES: REPORT FROM THE MYSTIC PROGRAM (UNITED STATES, 2003)

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MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

ABSTRACT

Background: Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program is a longitudinal global surveillance network of medical centers that utilize carbapenems (CARB) to treat serious infections. Currently 15 medical centers participate in the United States which includes 6 of the original 10 sites from 1999. We evaluated the influence of clonal dissemination on the antimicrobial resistance rates.

Methods: Each center submits to a central processing laboratory up to 200 aerobic bacterial isolates per year. Over 13,000 bacterial strains have been tested for susceptibility (1999-2003) against 12 antimicrobial agents using NCCLS methods and interpretive criteria. Selected isolates with resistant (R) antibiograms were genotyped for clonality by ribotyping (Riboprinter) and pulsed-field gel electrophoresis when necessary.

Results: Center 01 showed the highest rate of fluoroquinolone (FQ)-R among *E. coli* (68%) and 6 ribogroups were identified among the 7 FQ-R isolates (only one clone). Among *Klebsiella* spp. isolates, clonality was observed for both ESBL-producing and non-producing isolates. Five multi-drug resistant (MDR) *A. baumannii* (ACB) isolates from site 11 showed an identical ribogroup (192.1). These isolates were CARB-susceptible (S) and their ribogroup has not been identified previously in the program (unique). Five of 6 MDR-ACB strains from another medical center (04) were identical. These isolates were CARB-non-S and had the same ribogroup (931.7) previously reported in 3 other sites in the same geographic area (New York, Delaware) and 1 remote site in Colorado.

Conclusions: Our results indicated that the dissemination of R clones had an important influence on the CARB-R rates among ACB. The influence of clonality was less significant among other R patterns evaluated, such as ESBL-producing *Klebsiella* spp. and FQ-R *E. coli*. Surveillance networks like the MYSTIC Program are necessary to monitor the spectrum and continued potency of broad-spectrum agents, and has documented the spread of R clones within bacterial species that influence rates of R unrelated to local drug use.

INTRODUCTION

The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program is a global, longitudinal antimicrobial resistance surveillance program initiated in 1999 in the United States (US) to perform post-market surveillance and monitor the continued potency and spectrum of meropenem. Regional monitoring is performed in Europe, North America, Latin America, and the Asia-Pacific with greater than 125 participating medical centers, representing more than 30 countries. MYSTIC Program participant sites are medical centers actively utilizing carbapenems for the treatment of serious infections. In the US, participants forward bacterial isolates to a central processing laboratory along with limited demographic and local susceptibility testing data.

In this study, we report on the clonal spread within centers for strains of *Escherichia coli, Klebsiella* spp. and *Acinetobacter* spp. identified by antibiogram analysis during the processing of bacterial pathogens collected in the MYSTIC Program (2003). These susceptibility and clonality results can be used to assist the participant medical centers with empiric treatment choices and help define the need to promote epidemiologic intervention programs within their institutions.

MATERIALS AND METHODS

Participant sites. Fifteen medical centers, geographically distributed across the US, participated in the MYSTIC Program in 2003. All medical centers continued participation from the previous year and included six of the original 10 centers recruited in 1999.

MATERIALS AND METHODS CONTINUED

Bacterial isolates. Each center was requested to submit 140 Gram-negative and 60 Gram-positive aerobic strains isolated from serious infections to the central monitoring laboratory (JMI Laboratories, North Liberty, IA). Organism identifications were confirmed by colony morphology, biochemical tests and/or the Vitek System (bioMerieux, Hazelwood, MO). A total of 2,060 Gram-negative bacilli and 788 Gram-positive isolates were processed. All isolates were stored in an organism bank for molecular or epidemiologic investigations.

Susceptibility testing. Isolates were tested using National Committee for Clinical Laboratory Standards (NCCLS) reference methods. Susceptibility and resistance was determined by NCCLS interpretive criteria published in M100-S14. Quality control was assured by concurrent testing with American Type Culture Collection (ATCC) strains including *Enterococcus faecalis* ATCC 29212, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619.

Molecular testing. Resistant phenotypes within species groups and within participant sites were further characterized by antibiogram analysis. Groups of isolates with identical antibiograms were tested for genotypic relatedness by ribotyping using the Riboprinter™ Microbial Characterization System (Qualicon, Wilmington, DE) followed by pulsed-field gel electrophoresis (PFGE; BioRad Laboratories, Hercules, CA), as indicated.

RESULTS

- The ciprofloxacin susceptibility rate for *E. coli* at site 01 has consistently been lower than the annual aggregate rate for all sites, with a markedly reduced rate in 2003 (31.8%) compared to previous years (Table 1).
- Fifteen of the 22 *E. coli* isolates submitted from site 01 in 2003 were ciprofloxacin-resistant. Seven of these isolates (46.7%) demonstrated an identical antibiogram indicating a possible single clone. However, only two of the seven isolates shared the same ribotype (Table 2).
- Antibiogram and ESBL testing identified 12 *Klebsiella* spp. isolates from four sites to be tested for clonality. Four pairs of identical genotypes were discovered by ribotyping and PFGE (Table 2).
- Against *Acinetobacter* spp., the carbapenem susceptibility rate at site 04 remained much lower than the annual aggregate susceptibility rate. Isolates from site 11 demonstrated a much higher rate of carbapenem susceptibility (Table 1).
- Site 04 had a cluster of five carbapenem-resistant *A. baumannii* isolates with an identical genotype (931.7/A). This clone has been observed previously in this site and in other sites in the same geographic region (New York).
- Among the eight *Acinetobacter* spp. isolates from site 11, five demonstrated a common antibiogram (with meropenem MIC values of 4 or 8 g/ml), and an identical ribotype (192.1). Among those isolates, two PFGE types were identified: A and B (Table 2).

Table 1. Selected antimicrobial susceptibility trends in the MYSTIC Program (1999 - 2003).

Organism	Site	Antimicrobial agent	% susceptibility (number of isolates)[a] 1999	2000	2001	2002	2003
E. coli	01	Ciprofloxacin	70.0(20)	84.2(19)	60.0(20)	75.0(20)	31.8(22)
	All	Ciprofloxacin	95.9(197)	96.8(313)	90.5(306)	92.9(323)	89.1(469)
A. baumannii	04	Meropenem	40.0(5)	50.0(6)	40.0(5)	0.0(5)	40.0(10)
		Imipenem	40.0(5)	50.0(6)	40.0(5)	0.0(5)	70.0(10)
	11	Meropenem	[b]	100.0(5)	80.0(5)	100.0(5)	87.5(8)
		Imipenem	[b]	100.0(5)	80.0(5)	100.0(5)	100.0(8)
	All	Meropenem	78.1(32)	78.6(56)	81.0(79)	84.1(69)	87.4(111)
		Imipenem	81.2(32)	80.4(56)	83.5(79)	88.4(69)	91.9(111)

a. Criteria as published by the NCCLS [2004].
b. Site not tested in 1999.

Table 2. Molecular typing results for 30 antimicrobial-resistant isolates suspected to be clonally related in the MYSTIC Program (2003).[a]

Organism	Site	Isolate no.	Ribotype	PFGE
E. coli	01	2104	193.1	
		2276	193.2	
		2491	193.3	
		2538	1378.7	
		2653	243.2	
		2546	193.5	NT
		2656	193.5	NT
K. oxytoca	16	832	194.7	
		833	194.8	
K. pneumoniae	04	11	129.1	A
		68	129.1	A
		101	511.1	B
		129	511.1	B
		156	123.3	
	18	525	192.6	A
		528	192.6	A1
		530	192.8	
	21	683	183.6	A
		684	183.6	A
A. baumannii	04	66	931.7	A
		67	931.7	A
		73	931.7	A
		104	931.7	A1
		155	931.7	A1
		75	194.5	
	11	1143	192.1	A
		1144	192.1	A
		1147	192.1	B
		1149	192.1	B1
		1150	192.1	B

a. Strains indicated within boxes were clonally related by epidemiological tests (ribotyping and PFGE).

CONCLUSIONS

- Clonal dissemination has contributed significantly to elevated resistance rates in some MYSTIC Program institutions, especially for the organism groups such as *A. baumannii* and *K. pneumoniae*.
- Increasing carbapenem resistance among *Acinetobacter* spp. is mainly due to the clonal dissemination of resistant strains.
- Increasing fluoroquinolone resistance among *E. coli* was mainly due to wide selection of unique resistant mutants.
- Continued annual surveillance to include epidemiologic analysis with molecular methods to monitor for clonality remains necessary to accurately detect changing patterns of susceptibility within the carbapenem class.

MYSTIC PROGRAM SITES

Arkansas Children's Hospital (R Jacobs/T Beavers-May); Christiana Care (L Steele-Moore); Columbia Presbyterian Medical Center (P Della-Latta); Creighton University, St. Joseph Hospital (S Cavalieri/M Hostetter); Denver Health Medical Center (M Wilson/A Graepler); Iowa Methodist Medical Center (A Herring/L Roller); Kaiser Permanente Medical Group (J Fusco); Ochsner Clinic Foundation (G Pankey/D Ashcraft); University Hospitals of Cleveland (M Jacobs/S Bajaksouzian); University of Kentucky Hospital (J Ribes/S Overman); University of Texas, MD Anderson Cancer Center (K Rolston/R Prince); University of Utah, ARUP Laboratories, Inc. (A Croft); Veterans' Affairs Medical Center, Portland (D Sewell); Vanderbilt Medical Center (C Stratton/R Verrall); and Winthrop University Hospital (P Schoch).

SELECTED REFERENCES

Gales AC, Biedenbach DJ, Winokur P, Hacek DM, Pfaller MA, Jones RN. (2001). Carbapenem-resistant *Serratia marcescens* isolates producing Bush group 2f (SME-1) in the United States: Results from the MYSTIC Program. *Diagnostic Microbiology and Infectious Disease* 39:125-127.

Jones RN, Masterton R. (2001). Determining the value of antimicrobial surveillance programs. *Diagnostic Microbiology and Infectious Disease* 41:171-175.

National Committee for Clinical Laboratory Standards. (2003). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A6*. Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2004). *Performance standards for antimicrobial susceptibility testing. Supplemental tables M100-S14*. Wayne, PA:NCCLS.

Pfaller MA, Jones RN. (2000). MYSTIC (Meropenem Yearly Susceptibility Test Information Collection). Results from the Americas: Resistance in the treatment of serious infections. *Journal of Antimicrobial Chemotherapy* 46:25-37.

Pfaller MA, Jones RN, Biedenbach DJ, MYSTIC Study Group (USA). (2001). Antimicrobial resistance trends in medical centers using carbapenems: Report of 1999 and 2000 results from the MYSTIC Program (USA). *Diagnostic Microbiology and Infectious Disease* 41:177-182.

Rhomberg PR, Jones RN, The MYSTIC Program (USA) Study Group. (2003). Antimicrobial spectrum of activity for meropenem and nine broad spectrum antimicrobials: Report from the MYSTIC Program (2002) in North America. *Diagnostic Microbiology and Infectious Disease* 47:365-372.