72

Molecular Findings from SENTRY, a Global Antimicrobial Surveillance Program, in the Year 2001

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IDSA 2002

AMENDED ABSTRACT

Background: SENTRY is a global antimicrobial surveillance program that monitors isolates from US, Canada, Latin America (LA) & Europe (EU). A molecular typing protocol has been in place to monitor emerging or endemic resistances in participating medical centers.

Methods: Isolates with similar resistance (R) profiles & patient demographics or those islates exhibiting emerging R phenotypes are assessed. Species identification & antibiogram are confirmed. Isolates are then analyzed using an automated Riboprinter Microbial Characterization system (Qualicon, Inc). Isolates with identical patterns are further characterized using PFGE. Same Ribotype (RT) & identical/similar (≤ 3 bands difference) PFGE pattern is regarded as a cluster.

Results: During 2001 we characterized 647 isolates using RT & 468 by PFGE. A large fraction of the isolates was *S.aureus* with multi-drug-resistant, mupirocin- or Synercid-resistant phenotypes isolated from blood cultures, pneumonia & ICU patients. A total of 52 clusters were identified from 31 medical centers. Five RT were commonly observed and some of them were widespread around the globe (184-4) while others were restricted to one geographic area (893-2). Carbapenem-resistant *P. aeruginosa* (PSA) & *Acinetobacter* spp. (ACB) were also analyzed and some of which were metallo-ß-lactamase (MBL) producers. ACB MBL clusters were identified in Argentina & Israel while a PSA MBL epidemic strain was found in Italy (3 sites). Sites in Spain and US shared an ACB RT strain (1502-1). A total of 23 outbreaks /clusters were found among enteric bacilli showing ESBL & FQR phenotypes. *E. coli* RT were unique to the geographic locations while 3 *K. pneumoniae* RT were common between the US, LA & EU. Two *Salmonella* and 1 Grp. B Strep outbreaks were identified.

Conclusions: Increased usage of antimicrobials & travel trends has led to dissemination of R phenotypes between various geographic locations. Our molecular typing protocol has proven very effective in tracking these changing trends & distribution of antibiotic resistances around the globe & within local institutions.

INTRODUCTION

Global antimicrobial surveillance programs such as SENTRY play an important role in maintaining current knowledge on trends in antibiotic resistance in different geographic locations. All these programs utilize one or more susceptibility testing methods which provide a phenotypic profile of the response of microbial pathogens to an array of antimicrobial agents. This information is helpful in detecting certain resistance profiles and selecting potentially useful therapeutic agents. These methods are however not capable of tracing the spread of individual strains within a medical center or a geographic region. To obtain this epidemiological insight on the volumes of data generated in the surveillance programs, it is important to couple susceptibility testing with molecular epidemiological tools. SENTRY uses ribotyping and pulsed field gel electrophoresis (PFGE) for its primary molecular typing protocol to monitor emerging or endemic resistance profiles in participant medical centers.

Emerging resistance mechanisms among common pathogens such as ESBL-producing enterobacteriaceae, multi-drug resistant *S. aureus*, carbapenem-resistant and metallo-ß-lactamase producing *Pseudomonas* and *Acinetobacter* were targeted for molecular typing rather than seeking benign susceptible clusters that commonly occur in the hospitals.

MATERIALS AND METHODS

In SENTRY Antimicrobial Surveillance Program isolates from patients with blood stream infections, pneumonia, gastroenteritis, infections with ß-hemolytic streptococci and from those hospitalized in intensive care units were referred to the monitor center in Iowa. The identification on the isolates was confirmed to species level and they were tested against 30-40 key antimicrobials by reference dilution method (NCCLS, 2000). Patient demographic information was also collected on each isolate. Upon reviewing the data, possible clusters of isolates with similar resistance profiles and demographics were assessed. In the year 2001 of the SENTRY Program, 647 isolates were screened for potential clusters by ribotyping and 468 were subjected to PFGE.

Ribotyping: Potential clusters were subjected to ribotyping using automated Riboprinter Microbial Characterization System (Qualicon, Inc.). In short the isolates were grown overnight on BHI agar (Difco) and harvested in sample buffer. Genomic DNA was isolated and digested using *Eco*RI (all species except *Pseudomonas* spp. for which *Pvu*II was used). DNA was separated by agarose gel electrophoresis. Southern hybridization using probes derived from *E.coli* rRNA operon formed characteristic band patterns. These patterns were matched to pre-existing patterns by computer analysis and those with ≥ 93% identity was assigned to the same ribogroup. Isolates from the same medical center, isolated in a close time frame and with matching ribogroups were analyzed by PFGE. Isolates with different ribotypes were not evaluated further.

PFGE: Bacterial cells grown overnight were embedded in agarose, lysed and deproteinated to isolate near intact genomic DNA. The DNA was digested with an infrequently cutting restriction endonuclease appropriate for the species. The restriciton fragments were separated by electrophoresis on the CHEF DR II (BioRad, Hercules, CA) with the following conditions: 1% agarose, 0.5 X TBE, 200V for length of time and switch interval standardized for the species being tested. Ethidium bromide stained gels were examined visually. Isolates showing ≤ 3 bands difference were considered identical or similar clonally related.

RESULTS

S. aureus

- *S.aureus* ranked highest in occurrence, more than one-third of all isolates ribotyped belonged to this species. Fifty-two clonal outbreaks from 31 medical centers were confirmed. (Table 1, Table 2)

- Five major ribogroups dominated *S.aureus* population constituting 56% of all the isolates; three types were unique to a continent and two ribotypes overlapped (Table 3).

Non-fermentative gram-negative pathogens

- A large number of multi-drug resistant non-fermentative pathogens were encountered (*Pseudomonas* and *Acinetobacter*), many of which were metallo-ß-lactamase (MBL) producers.

- Multidrug resistant (MDR) or MBL-producing *P. aeruginosa* were more frequently isolated from Latin America and Europe. MBL and non-MBL (carbapenem-resistant) isolates often shared the same ribotype. Carbapenem-resistant *Pseudomonas aeruginosa* isolates with MBL phenotype and without MBL phenotype having the same molecular type were identified (Table 4).

- Clonal outbreaks with MBL-producing *P.aeruginosa* strains were detected in 3 Italian sites.

- Two sites in Latin America and Europe shared the same *P.aeruginosa* ribotype and similar PFGE pattern. Many MBL producers showed unique ribotypes (data not shown).

- Carbapenem-resistant *P. aeruginosa* and *A. baumannii* outbreaks in the USA were from a site in New York (NY). A site in Argentina and one in Israel showed a cluster of MBL-producing *A. baumannii*.

- Latin America and Europe showed a high prevalence of *Acinetobacter* spp. outbreaks (Table 5). No ribotypes were shared except for one type between NY and Spain (1502.1).

Table 1. Molecular analysis of multi-drug resistant organism clusters from the SENTRY Antimicrobial Surveillance Program, 2001.

Organism	No. isolates	No. MDR[a]	No. of sites with possible MDR clusters[b]	No. MDR clusters/possible clonal spread	No. of sites with MDR clones[c]
S. aureus	4650	236/267	34	52	31
K. pneumoniae	1348	110/254	24	24	18
E. coli	3314	33/119	12	4	4
P. aeruginosa	1954	96/231	11	15	9
Acinetobacter spp.	577	45/82	8	8	7
S. maltophilia	339	18/27	3	1	1
E. aerogenes	247	4/12	2	1	1
E. cloacae	712	21/58	9	5	5
Enterobacter spp.	1044	27/73	12	7	7
E. faecium	332	10/64	3	2	2
E. faecalis	907	5/13	2	1	1
Salmonella spp.	745	13/58	3	3	3
Coag-neg staph	1857	5/15	2	2	2
Group B strept	694	12/12	1	1	1
H. influenzae	2600	2/9	1	1	1

a. Number of MDR ribotypes/total number of MDR isolates
b. Sites from which isolates were ribotyped.
c. Sites which showed presence of clusters as documented by ribotyping and PFGE.

Table 2. Distribution of *S. aureus* clusters among sites monitored by the SENTRY Antimicrobial Surveillance Program.

Country/nation	Site #	# of clusters	Ribotype(s)
USA	001	1	184.4
	004	4	184.4, 266.4, 1280.6
	011	1	1511.1
	012	1	184.4
	013	1	184.4
	014	3	184.5, 371.1, 1511.1
	015	6	804.3, 184.5, 266.4, 910.8
	021	1	371.1
	024	2	184.4
	027	1	266.4
	030	1	266.4
	051	1	764.4
	055	2	1140.7, 36.2
	082	1	184.4
Latin America	039	2	893.2
	040	1	51.2
	042	2	893.2
	043	2	893.2
	046	1	893.5
	048	1	904.1
	057	2	893.5
	101	2	893.5
Europe	058	1	371.1
	061	2	184.5
	063	2	268.6, 1495.4
	069	2	347.2
	075	1	686.4
	077	2	653.5, 371.2
	081	1	184.5
	091	1	184.5
	096	1	901.4

- Only one *S. maltophilia* outbreak was documented from Canada involving 11 isolates from pneumonia patients (Table 1).

Enterobacteriaceae

- *K.pneumoniae* ranked second overall. Although more *E.coli* isolates were processed, more strains of *K. pneumoniae* showed the ESBL phenotype and confirmed clonal outbreaks (Table 1). Twenty-four clusters of *K.pneumoniae* were encountered versus only four for *E.coli*.

- A larger number of ESBL-producing *K.pneumoniae* clusters were noted in Europe, as well as three each in the USA and Latin America (Table 6)

- All *K.pneumoniae* ribotypes were restricted to the nation or continent of origin except one ribotype shared between the USA and Europe (511.3), but their PFGE patterns did not match.

- Latin America and Europe dominated in other Enterobacteriaceae outbreaks, as listed in Table 7.

Other pathogens

- Three vancomycin-resistant enterococcal outbreaks were discovered, two from the USA and one from Brazil (Table 8).

- Two group ß-streptococcus outbreaks were seen from a site in Brazil (Table 1).

Table 3. Geographic distribution of the five most commonly seen *S. aureus* ribotypes in SENTRY, 2001.

Ribotype	No. isolates	Occurrence # of centers	Country/nation(s)
893.5	39	7	Latin America
184.4	32	14	USA
184.5	23	9	USA, Europe
893.2	22	4	USA, Latin America
266.4	14	6	USA

Table 4. Distribution of metallo-ß-lactamase producing or multi-drug resistant[a] *P. aeruginosa* clusters.

Country/nation	Site #	Ribotype	# of clusters
USA	015	21.3	1
Latin America	042	22.1	1
	046	100.5, 47.1	2
	048	559.4, 60.6	2
	101	559.4, 1423.6	3
Europe	061	676.8	1
	063	1034.2	1
	068	798.3, 1034.2	1
	075	1034.2, 44.1, 44.2	3

a. Multi-drug resistance defined as resistance to ceftazidime, gentamicin, ciprofloxacin, and piperacillin/tazobactam.

Table 5. Occurrence of *Acinetobacter* outbreaks among SENTRY sites.

Country/nation	Site #	R phenotype	Ribotype	# clusters	# isolates
USA	015	Carbapenem	1502.1	1	6
Latin America	039	Carbapanem, MBL[a]	647.3	1	8
		MDR[a]	1736.8	1	2
	049	MDR[a]	1745.4	1	4
	057	OUTB	56.7	1	2
Europe	063	Carbapenem, MBL[a]	1222.6	1	4
	065	Carbapenem	1502.1	1	4
		Carbapenem, MBL[a]	218.3	1	5
	081	MDR[a]	531.3	1	2

a. MDR = multi-drug resistant; MBL = metallo-ß-lactamase; OUTB = outbreak

Table 6. Clonal outbreaks of extended spectrum ß-lactamase producing *K. pneumoniae* isolates in the year 2001.

Country/nation	Site #	Ribotype	PFGE	PFGE comparison[a]	# isolates
USA	014	511.8	14A	Similar to 52A	2
	015	1011.4	15B	-	2
		30.5	15A	Similar to 48A	2
	052	511.3	52A	Similar to 14A	2
Latin America	039	713.4	39A	-	3
	048	30.5	48A	Similar to 15A	2
	057	252.4	57A	-	2
		520.4	57B	-	3
Europe	062	21.1	62A	-	3
		1175.7	62B	-	2
		556.7	NT[b]	-	3
	063	598.1	63A	-	4
	066	17.5	66A	-	3
	069	1143.3	69A	-	2
	077	203.3	77B	Not similar to 85B	2
	081	830.6	81A	Similar to 85A	2
	085	830.6	85A	Similar to 81A	2
		203.3	85B	Not similar to 77B	2
		511.3	85C	Not similar to 14A	2
	086	262.1	86A	-	4
	091	614.4	91A	-	5
	092	614.4	92A	Not similar to 91A	3
	095	203.3	95A	Not similar to 77B	2
	097	511.1	97A	-	2

a. PFGE patterns compared across sites when ribotypes matched.
b. NT = not typeable by PFGE.

Table 7. Distribution of Enterobacteriaceae clones other than *K. pneumoniae*.

Country	Site #	Organism	R phenotype	Ribotype	PFGE	# isolates
US	065	*S. marcescens*	AMP-C[a]	65.8	39A	2
Latin America	039	*P. mirabilis*	ESBL	65.8	39A	2
	040	*E. cloacae*	ESBL	870.2	40A	3
	042	*E. coli*	ESBL	15.3	42A	3
		P. mirabilis	ESBL	618.6	42A	2
	043	*E. cloacae*	ESBL	539.6	43A	4
	046	*S. marcescens*	ESBL	191.2	46A	3
	048	*Salmonella* spp.	FQR[a]	14.1	48A	3
	101	*E. cloacae*	ESBL	96.1	101A	2
Europe	066	*E. coli*	FQR[a]	253.3	Pending	4
		E. gregoviae	OUTB[a]	17.7	66A	2
	073	*E. aerogenes*	ESBL	226.3	73A	2
	075	*Salmonella gr. C*	FQR[a]	64.7	75A	2
	081	*E. cloacae*	ESBL	512.3	81A	2
	085	*E. coli*	ESBL	750.7	85A	2
	086	*E. coli*	ESBL	254.2	Pending	2
				241.4	Pending	3
	091	*S. typhimurium*	OUTB[a]	15.4	91A	8
	097	*P. mirabilis*	ESBL	64.5	97A	2

a. AMP-C = Amp C phenotype; FQR = fluoroquinolone-resistant; and OUTB = outbreak.

Table 8. Outbreaks caused by *Enterococcus* spp. in the year 2001.

Country/nation	Site #	Species	R phenotype	Ribotype	# isolates
US	011	*E. faecium*	VRE/1 LINR[a]	1389.6	4
	052	*E. faecium*	VRE[a]	187.4	2
Latin America	048	*E. faecalis*	VRE[a]	30.1	3

a. LINR = Linezolid resistant, one isolate. VRE = vancomycin-resistant *Enterococcus* spp.

CONCLUSIONS

- *S.aureus* is the predominant pathogen among blood stream infections, pneumonia and ICU patients, and additionally caused the most clonal outbreaks in all SENTRY Program monitored continents except Canada.
- Distinct geographic distributions of ribotypes were observed.
- Clonal outbreaks of MBL-producing isolates were on the rise in Europe and Latin America.
- There is a very high prevalence of ESBL-producing or other multi-drug resistant Enterobacteriaceae pathogens in Europe.
- In *S. aureus* and *P.aeruginosa* isolates, it was common to observe the same ribotype exhibiting different PFGE patterns. In most other Gram-negative pathogens the ribotyping and PFGE results were complimentary.
- Our protocol of molecular typing has proven advantageous and effective in tracking antimicrobial-resistant isolates for this longitudinal global antimicrobial surveillance program.
- Ribotyping and PFGE were complimentary for molecular epidemiological purposes, accurately detecting dozens of epidemic occurrences in 2001.

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