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MOLECULAR DETERMINATION OF β-LACTAMASE TYPES FOUND IN *SALMONELLA* SPP. ISOLATED IN INDIA: REPORT FROM THE MYSTIC PROGRAM (2001)

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MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

INTRODUCTION

Resistance mechanisms among Gram-negative bacilli have increased over the last 2 decades. Extended-spectrum β-lactamase enzymes (ESBLs) have challenged the clinical utility of 'third-generation' cephalosporins and monobactams among commonly isolated Enterobacteriaceae. Variations regarding the prevalence of ESBL-producing strains of Enterobacteriaceae have been reported with particularly high rates noted in Latin America, portions of Europe, and the Asia-Pacific region. However, some nations do not have structured resistance surveillance programs to determine the accurate prevalence of β-lactam resistance among key pathogens. India has reported limited information regarding nationwide resistance rates among clinical isolates including *Salmonella* spp. which are common bacteremic pathogens in this region of the world.

Reports dating from 1996 describe third-generation cephalosporin resistance rates in India at 25-65% for *Klebsiella pneumoniae*. *Pseudomonas aeruginosa* isolates resistant to ciprofloxacin were reported to be 60%. These results create increasing concern that the use of sub-standard products and/or underdosing may cause resistance selection, and that transmission of resistant phenotypes may be due to infection control deficiencies in some institutions. If these resistance problems were to be substantiated, the use of broader-spectrum agents, such as meropenem, in conjunction with improved infection control practices, may be necessary in institutions where resistance rates are high.

METHODS

Strains were selected from isolates obtained in 10 medical centers during 2000. Approximately 5 strains were to be collected from each of the following institutions: Bangalore (1 site), Indore (1 site), Lucknow (1 site), Mumbai (2 sites), New Delhi (4 sites), and Vellore (1 site). From a total of 57 isolates, 21 strains with suspected β-lactamase (BL) enzymes were chosen for further genotypic evaluation. All strains were isolated from significant infections in hospitalized patients.

Initial testing was performed by the Etest® method (AB BIODISK, Solna, Sweden), focusing on 6 β-lactam drugs (cefotaxime, cefpirome, ceftazidime, imipenem, and piperacillin with and without tazobactam). Strains were retested in 2001 with 10 drugs (meropenem, cefepime, ceftazidime, ceftizoxime, ceftriaxone, aztreonam, piperacillin/tazobactam, ciprofloxacin, gentamicin, and tobramycin) using the NCCLS broth microdilution method. The definitions of an ESBL phenotype were those of the NCCLS, and each isolate was retested using selected β-lactam substrates (cefepime, cefotaxime, and ceftazidime) with added clavulanic acid.

Strains with a MIC at ≥2 µg/ml for aztreonam, cefotaxime, ceftriaxone or ceftazidime, were tested (Etest) for a reduction in the MIC (≥3-fold) which is a confirmation of an ESBL phenotype. Strains having similar quantitative antibiograms were then processed by molecular typing methods that included automated ribotyping and pulsed-field gel electrophoresis (PFGE). All strains with a piperacillin MIC at ≥32 µg/ml were screened for the presence of BL enzymes. For these isolates, encoding genes were aligned and generic primer sets constructed confirming sequence data was performed by DuPont Automated Systems analyzed by DNAstar.

RESULTS

- Invasive bloodstream infection isolates of *Salmonella* spp. generally remained susceptible (>94.7%) to β-lactam antibiotics, fluoroquinolones and aminoglycosides in India (Table 1).
- The potency of cephalosporins and ciprofloxacin ($MIC_{s_{90}}$, 0.06-0.5 µg/ml; % susceptibility 94.7-96.5) was greater than piperacillin/tazobactam (MIC_{90}, 2 µg/ml; % susceptibility 94.7), but meropenem was the most potent agent tested overall (MIC_{90}, 0.03 µg/ml; % susceptibility 100.0) and inhibited all isolates at ≤0.06 µg/ml.
- Among the *Salmonella* spp. tested, 3 strains had MICs to ceftazidime, ceftriaxone or aztreonam consistent with NCCLS ESBL criteria (≥2 µg/ml). These strains were resistant to all third-generation cephalosporins, aztreonam and aminoglycosides. Only 1 isolate had a reduction in the ceftazidime MIC in the presence of clavulanic acid (positive Etest ESBL result; CTX-M15).
- Between 1 and 4 BL enzymes were found in each strain screened. TEM-1 was very common in all medical centers while SHV-1 was found in only 3 isolates from 2 medical centers. OXA-1 and -2 primer set-type enzymes were also fairly common.
- CMY-6 and CMY-7 enzymes were observed in 2 multidrug-resistant (MDR) strains from different medical centers and geographic areas.
- No clonal dissemination of MDR *Salmonella* spp. invasive isolates was detected in molecular typing methods.

Table 1. Activity and spectrum of meropenem compared with 9 other antimicrobial agents tested against invasive isolates of *Salmonella* spp. from India

		MIC (µg/ml)			% by category[a]	
Organism (no. tested)	Antimicrobial agent	50%	90%	Range	Susceptible	Resistant
Salmonella spp. (57)	Meropenem	≤0.016	0.03	≤0.016-0.06	100.0	0.0
	Ceftazidime	0.25	0.5	≤0.12->16	94.7	5.3[b]
	Ceftizoxime	0.03	0.06	≤0.016->32	94.7	5.3
	Ceftriaxone	0.12	0.12	0.06->32	94.7	5.3[b]
	Cefepime	≤0.12	≤0.12	≤0.12->16	96.5	1.8
	Aztreonam	≤0.12	0.25	≤0.12->16	94.7	5.3[b]
	Piperacillin/tazobactam	0.5	2	≤0.25-128	94.7	5.3
	Ciprofloxacin	≤0.25	0.5	≤0.25->2	96.5	3.5
	Gentamicin	≤2	≤2	≤2->8	94.7	5.3
	Tobramycin	≤1	≤1	≤1->8	94.7	5.3

[a] Susceptibility criteria of the NCCLS (2002).
[b] Three strains achieved the criteria of an ESBL phenotype (NCCLS, 2002), one was inhibited by 2 µg/ml of clavulanate (confirmation test-positive).

Table 2. Variations of ESBL phenotypes and β-lactamase-producing strains found among isolates of *Salmonella* spp. during a surveillance trial in India (2000)

			β-lactamase type							
Medical center/ strain	ESBL phenotype/ Etest[a]	No. β-lactamases	TEM-1	SHV-1	CTX-M	CMY	OXA-1	OXA-2	Co-R[b]	Clonal cluster[c]
A6/1	+/-	1	ND	ND	-	CMY-7	-	-	GC	No
B6/1	-/ND	1	ND[d]	-	-	-	-	-		
B6/4	-/ND	1	+	ND	ND	ND	ND	ND		
B6/5	-/ND	1	+	ND	ND	ND	ND	ND		
B6/10	-/ND	2	+	-	-	-	-	+		
G6/9	+/-	4	-	+	-	CMY-6	+	+	GC	No
G6/11	-/ND	1	+	-	-	-	-	-		
G6/13	-/ND	1	+	-	-	-	-	-		
H6/1	-/ND	1	+	-	-	-	-	-		
H6/2	-/ND	1	+	ND	-	-	-	-		
H6/7	-/ND	1	+	-	-	-	-	-		
H6/8	-/ND	2	+	+	-	-	-	-		
H6/15	-/ND	2	+	-	-	-	-	+		
H6/20	-/ND	3	+	+	-	-	-	+		
H6/9	-/ND	2	+	-	-	-	+	-		
K6/9	+/+	4	+	-	M-15	-	+	+	GC	No
J6/9	-/ND	1	+	-	-	-	-	-		
J6/12	-/ND	1	+	-	-	-	-	-		
J6/14	-/ND	2	+	-	-	-	-	+		
J6/15	-/ND	1	+	-	-	-	-	-		
J6/16	-/ND	1	+	-	-	-	-	-		

[a] Etest ESBL test used to confirm the presence of an inhibitable (clavulanate) β-lactamase.
[b] Co-R = co-resistance including gentamicin (G) and/or ciprofloxacin (C).
[c] Clonal relationships were assessed by automated ribotyping and PFGE.
[d] ND = not determined.

CONCLUSIONS

- **Among isolates of *Salmonella* spp. from hospitals in India, meropenem maintains complete (100% susceptible) activity although most broad-spectrum β-lactams, quinolones and aminoglycosides also retain (≥94.7%) activity against these strains.**
- **The frequency of BL-producing isolates of *Salmonella* spp. in India is high and can compromise the activity of β-lactams, fluoroquinolones and aminoglycosides; the latter 2 of which are common co-resistant determinants among BL-producing isolates.**
- **The presence of TEM-1 β-lactamase was very common and OXA-1 and -2 primer-type enzymes were also frequent in some medical centers in India. The presence of CTX-M enzymes and CMY-type enzymes was noted among the isolates in this study including a previously unreported CMY-7 enzyme in a *Salmonella* spp. isolated from India.**

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