# INITIAL STUDIES OF RESISTANCE PATTERNS IN INDIA: MEROPENEM YEARLY SUSCEPTIBILITY TEST INFORMATION COLLECTION (MYSTIC) PROGRAM BASELINE RESULTS FOR MULTI-RESISTANT GRAM-NEGATIVE BACILLI AND ENDEMIC SALMONELLA SPP. ISOLATES

RN Jones, D Mathai, PR Rhomberg and the MYSTIC Advisory Board

The JONES Group/JMI Laboratories, North Liberty, IA, USA; and the Christian Medical College Hospital, Vellore, India

MYSTIC
Meropenem Yearly Susceptibility Test Information Collection

## INTRODUCTION

Numerous novel resistance mechanisms have emerged among Gram-negative bacilli in the past two decades. Extended-spectrum β-lactamases (ESBLs) have challenged the continued clinical utility of the third-generation cephalosporins and monobactams, especially those found in *Escherichia coli* and *Klebsiella* spp.. Wide variations have been observed in the prevalence of ESBL-producing strains within and between geographic areas. High ESBL occurrence rates have particularly compromised therapy in Latin America, portions of Europe and the Asia-Pacific region, yet in some nations structured resistance surveillance programs have not clarified the accurate prevalence. An example of a country with a great population of patients is India, where limited information has been reported about nationwide resistance rates among common clinical isolates.

Brief communications dating from 1996 describe resistance rates in India as 25-65% for *K. pneumoniae* when tested against third-generation cephalosporins, and *Pseudomonas aeruginosa* resistance to ciprofloxacin was described at 60%. These results were of great concern and a contributing factor may be the use of substandard products leading to underdosing and resistance selection. If these resistance problems were substantiated, the use of broader-spectrum agents, such as the carbapenems (e.g. meropenem), may be necessary in those institutions in India where the resistance rates are most elevated. In late 2001, a group of Indian microbiologists (from 10 medical centers) reported alarmingly high ESBL rates in *E. coli* (61%) and *Klebsiella* spp. (57%), and other significant resistance patterns in nonfermentative and enteric Gram-negative bacilli. In that study the most active agents tested were the carbapenems.

Meropenem has been projected for introduction into India in 2002 as an alternative treatment for multi-resistant organisms. To evaluate its potential role, the most resistant Gram-negative bacilli, as well as isolates from *Salmonella* spp. bacteremia, from the 10-center trial were retested by reference broth microdilution methods (NCCLS) against meropenem and 10 comparator broad-spectrum drugs.

## MATERIALS & METHODS

### *Organisms tested*

The strains were selected from the collection obtained in the 10 medical center study performed in 2000-2001. The study sampled approximately 100 strains from each institution including those in Bangalore (one site), Mumbai (two), New Delhi (four), Lucknow (one), Indore (one) and Vellore (one). From a total of 854 validated strains, 212 isolates with unusually high multidrug resistance (MDR) rates were chosen as well as a geographically diverse series of *Salmonella* spp. isolates from bloodstream infections. All strains were from significant infections in patients from each medical center.

### *Susceptibility test methods*

Initial testing was performed in 2000-2001 by the Etest (AB BIODISK, Solna, Sweden) focusing on β-lactam drugs (imipenem, cefotaxime, cefpirome, ceftazidime, piperacillin with and without tazobactam). Selected strains were retested with the NCCLS broth microdilution method using 11 drugs: meropenem, imipenem, cefepime, ceftazidime, ceftizoxime, ceftriaxone, aztreonam, piperacillin/tazobactam, ciprofloxacin, gentamicin and tobramycin. The definitions of an ESBL phenotype were those of the NCCLS and all were retested with selected β-lactam substrates (cefepime, cefotaxime, ceftazidime) with added clavulanic acid. Strains (1) having similar quantitative antibiograms with the 11 drugs; (2) isolated from the same institutions and time frame; and (3) having the same ESBL resistance phenotype, were then processed by molecular typing methods that included automated ribotyping and pulsed-field gel electrophoresis (PFGE).

## RESULTS

- Characteristics of the 212 strains are summarized in Table 1. A total of 125 strains (59.0%) were confirmed ESBL-producing isolates, 111 (52.4%) in *E. coli* or *Klebsiella* spp..
- Among 57 *Salmonella* spp. strains (bacteremias), numerous isolates were resistant to older penicillins (ampicillin, data not shown) and only three ESBL phenotypes were identified. Two (3.9%) of these strains had enzymes inhibited by clavulanic acid. Meropenem was active against all *Salmonella* spp. strains ($MIC_{90}$, 0.03 µg/mL) and was superior to all other agents in potency and proportion of susceptible isolates (100% vs 94.7-96.5%, see Table 2).
- Among 71 *E. coli* strains with an ESBL phenotype, 65 had an inhibitable enzyme (Table 3). Meropenem inhibited all strains at ≤0.12 µg/mL ($MIC_{90}$, 0.06 µg/mL) and piperacillin/tazobactam had the next best spectrum at only 80.3%. All ESBL phenotypes of *E. coli* were ciprofloxacin-resistant, coming from all 10 monitored medical centers.
- In the *Klebsiella* spp. ESBL phenotypes, 46 of 48 strains were confirmed by clavulanate inhibition (Table 3), and again meropenem ($MIC_{90}$, 0.06 µg/mL; 100.0% susceptible) was the most active agent followed by piperacillin/tazobactam (62.5% susceptible) and ciprofloxacin (39.6% susceptible).
- Among 17 other MDR species strains of Enterobacteriaceae (Table 1), 13 had ESBL phenotypes and 12 were confirmed. Table 4 lists all 12 strains and illustrates possible clonal dissemination of an *Enterobacter* sp. (four isolates) in medical center F, and a *Providencia stuartii* (two isolates) in medical center B. Only meropenem was effective *in vitro* versus these epidemic strains.
- Meropenem was generally four-fold more active than imipenem (Figure 1) against all MDR challenge strains from the Indian hospitals, with a near complete spectrum (99.1% at ≤4 µg/mL). Only two strains (*Acinetobacter* sp. and *Pseudomonas putida*) had meropenem MICs at 8 µg/mL - an intermediate MIC result.

## CONCLUSIONS

- **Very high levels of β-lactam resistance (confirmed ESBL phenotypes) were documented in all 10 medical centers in India among several enteric bacilli (also *Salmonella* spp.).**
- **Co-resistances were very common with aminoglycosides and ciprofloxacin.**
- **The rank order of most active agents against these 212 isolates from India was: meropenem (99.1% susceptible) > piperacillin/tazobactam (76.9%) > ciprofloxacin (42.5%) > aminoglycosides (34.4-39.6%) = other β-lactams (30.0-39.6%).**
- **Meropenem should offer a welcome, broad spectrum of therapy versus resistant endemic and epidemic Gram-negative bacilli in the Indian medical centers monitored.**

## SELECTED REFERENCES

Arya SC. Emerging antibiotic Resistance in Indian communities. Clin Infect Dis 25: 944-945, 1997.

Expert Group on Antibiotic Susceptibility Tests (EGAST). Antimicrobial susceptibility of bacteria isolated from hospitalized patients with acute, serious infections (a multicenter study). Indian J Intern Med 5(Suppl 5): 36-44, 1995.

Jones RN, India Antimicrobial Resistance Study Group, Mathai D, Biedenbach DJ. Evaluation of six broad-spectrum β-lactams tested against recent clinical isolates from India: A 10 medical center survey, Abstr. C2-290. 41st ICAAC, Chicago, IL, USA, 2001.

Mehta A, Rodriguez C. Business India, August 14: 169-172, 1995.

National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard Document M7-A5. Wayne, PA: NCCLS, 2000.

National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing. Approved Standard Document M100-S12. Wayne, PA: NCCLS, 2002.

Peter JB. Antimicrobial resistance in India and the United States. Clin Infect Dis; 23: 1315-1316, 1996.

Srivestara L, Aggarwal P. Multidrug-resistant *Salmonella typhi* in Delhi. Indian J Med Microbiol 12: 102-105, 1994.

**Table 1. Listing of organisms tested and their resistance features (212 isolates from India, 2000-2001)**

| Organism | No. tested | ESBL[a] Phenotype | ESBL[a] Confirmed | Comment |
|---|---|---|---|---|
| *E. coli* | 74 | 71 | 65 | 2 susceptible controls were tested |
| *Enterobacter* spp. | 13 | 9 | 8 | All resistant to ≥3 antimicrobial classes |
| *Klebsiella* spp. | 53 | 48 | 46 | 3 susceptible controls were tested |
| *Salmonella* spp. | 57 | 3 | 2 | Prevalence sample, bacteremias |
| Other species[b] | 15 | 4 | 4 | Multidrug-resistant strains only |
| TOTAL | 212 | 135 | 125 | - |

[a]Phenotype = strains having a MIC of ≥2 µg/mL for aztreonam or ceftriaxone or ceftazidime; and confirmed = strains with phenotype and demonstrating a ≥eight-fold reduction of the MIC in the presence of clavulanic acid (2 µg/mL).

[b]Includes *Acinetobacter* spp. (four strains), *Citrobacter freundii* (two strains), *P. aeruginosa* (four strains), *Providencia stuartii* (two strains), and one strain each of *Pseudomonas. stutzeri*, *Pseudomonas* spp., NOS, and *Ralstonia pickettii*.

**Table 2. Activity and spectrum of meropenem compared to nine other antimicrobial agents tested against invasive isolates of *Salmonella* spp. (n=57) from India**

| Antimicrobial agent | MIC (µg/mL) 50% | 90% | Range | % by category[a] Susceptible | Resistant |
|---|---|---|---|---|---|
| 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.

[b]Three strains achieved the criteria of an ESBL phenotype, two were inhibited by 2 µg/mL of clavulanate (confirmation test - positive).

**Table 3. Antimicrobial activity of meropenem and nine comparison broad-spectrum agents tested against 119 strains of *E. coli* and *Klebsiella* spp. having a positive ESBL resistance screening test[a]**

| Organism (no. tested) | Antimicrobial agent | MIC (µg/mL) 50% | 90% | Range | % by category[a] Susceptible | Resistant |
|---|---|---|---|---|---|---|
| *E. coli* (71) | Meropenem | 0.03 | 0.06 | ≤0.016-0.12 | 100.0 | 0.0 |
| | Ceftazidime | >16 | >16 | 2->16 | 7.0 | 52.1 |
| | Ceftizoxime | >32 | >32 | 4->32 | 8.5 | 81.7 |
| | Ceftriaxone[b] | >32 | >32 | >32 | 0.0 | 100.0[b] |
| | Cefepime | >16 | >16 | 8->16 | 2.8 | 84.5 |
| | Aztreonam[b] | >16 | >16 | 16->16 | 0.0 | 98.6[b] |
| | Piperacillin/tazobactam | 8 | 32 | 2->128 | 80.3 | 2.8 |
| | Ciprofloxacin | >2 | >2 | >2 | 0.0 | 100.0 |
| | Gentamicin | >8 | >8 | ≤2->8 | 12.7 | 87.3 |
| | Tobramycin | >8 | >8 | ≤1->8 | 4.2 | 95.8 |
| *Klebsiella* spp. (48) | Meropenem | 0.03 | 0.06 | ≤0.016-1 | 100.0 | 0.0 |
| | Ceftazidime | >16 | >16 | 8->16 | 8.3 | 72.9 |
| | Ceftizoxime | >32 | >32 | 8->32 | 2.1 | 70.8 |
| | Ceftriaxone[b] | >32 | >32 | 32->32 | 0.0 | 95.8[b] |
| | Cefepime | >16 | >16 | 1->16 | 8.3 | 85.4 |
| | Aztreonam[b] | >16 | >16 | 8->16 | 2.1 | 97.9[b] |
| | Piperacillin/tazobactam | 16 | 128 | 2->128 | 62.5 | 16.7 |
| | Ciprofloxacin | 2 | >2 | ≤0.25->2 | 39.6 | 35.4 |
| | Gentamicin | >8 | >8 | ≤2->8 | 8.3 | 91.7 |
| | Tobramycin | >8 | >8 | ≤1->8 | 6.3 | 93.7 |

[a]ESBL phenotype as defined by the NCCLS. A total of 111 strains (93.3%) had an enzyme inhibited by clavulanic acid (2 µg/mL), a positive confirmation result.

[b]The most enzyme-sensitive substrates for ESBL recognition in India.

**Table 4. Additional isolates of Enterobacteriaceae characterized as having an inhibitable (2 µg/mL clavulanate) ESBL by modified NCCLS (2002) criteria.[a] Twelve strains were tested from five species**

| Organism (no. tested) | Medical center | MIC (µg/mL) Meropenem | Ceftriaxone | Ceftazidime | Cefepime | P/T[b] | Tobramycin | Ciprofloxacin |
|---|---|---|---|---|---|---|---|---|
| *Enterobacter* spp. (8)[a] | A | 0.03 | >32 | >16 | 8 | 64 | >8 | ≤0.25 |
| | C | 0.03 | >32 | >16 | >16 | 64 | >8 | ≤0.25 |
| | E | 0.06 | >32 | >16 | >16 | 32 | >8 | ≤0.25 |
| | E | 0.06 | >32 | >16 | >16 | >128 | >8 | >2 |
| | Fc | 0.12 | >32 | >16 | >16 | 128 | >8 | 2 |
| | Fc | 0.12 | >32 | >16 | >16 | 128 | >8 | >2 |
| | Fc | 0.12 | >32 | >16 | >16 | 128 | >8 | 2 |
| | Fc | 0.12 | >32 | >16 | >16 | 128 | >8 | 2 |
| *Citrobacter freundii* (2) | E | 0.03 | >32 | >16 | >16 | 32 | >8 | >2 |
| | I | 0.03 | >32 | >16 | 8 | 4 | >8 | >2 |
| *P. stuartii* (2) | Bc | 0.03 | >32 | >16 | 16 | 2 | 8 | 2 |
| | Bc | 0.06 | >32 | >16 | >16 | 2 | 8 | 2 |

[a]Among nine strains of *Enterobacter* spp. with cefepime MICs at ≥8 µg/mL, eight contained an inhibitable ESBL by NCCLS criteria.

[b]P/T = piperacillin/tazobactam.

[c]Clonal occurrence confirmed by molecular epidemiologic typing that included PFGE and automated ribotyping.

**Figure 1. Comparisons of potency for two carbapenems (meropenem, imipenem) tested against the entire collection of 212 multidrug-resistant Gram-negative bacilli from India (2000-2001)**