codon83 (Serine83Arginine) was noted in 1 strain having a CIP MIC of 0.012 and a NA MIC of 32/μg/ml. All 28 strains that had a CIP MIC of 0.016–1.5/μg/ml and frank resistance to NA (MIC ≥16/μg/ml; DD zone of inhibition ≤13 mm) carried a transversion mutation in codon 83 of gyrA (Serine83Isoleucine). We noted another transversion mutation in codon 85 of parC (Serine85Leucine) in 14 of these 28 strains that had a CIP MIC of ≥0.125–1.5 and a NA MIC ≥256/μg/ml. We could not detect any mutations in gyrB (n = 24) and parE (n = 28) genes.

Conclusion: Reduced CIP-susceptibility and NA-resistance of V. cholerae is associated with a gyrA mutation (Serine83Isoleucine). A further decrease in CIP-susceptibility is associated with parC mutation (Serine85Leucine).

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17.019

Prevalence of Extended-Spectrum β-Lactamases produced by Klebsiella spp. from various clinical samples in an urban hospital in South India

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Background: Organisms producing extended-spectrum β-lactamases (ESBL) are emerging around the world as a source of resistance to oxyiminocephalosporins such as cefotaxime, ceftazidime and ceftriaxone. This study was undertaken to study the prevalence of ESBL production by Klebsiella spp. in an urban hospital in South India.

Methods: Antimicrobial susceptibility testing of 213 strains of Klebsiella spp., isolated from various clinical samples in an urban hospital in South India.

Results: Of the 105 strains screened for ESBL production 45 (42.85%) and 43 (40.95%) strains were positive by PCDDT and DDST methods respectively and 53 (50.47%) strains were positive by PCDDT method. The ESBL was identified by double-disk synergy test (DDST) and the phenotypic method include the PCR amplification method.

Conclusion: The prevalence of ESBL produced by Klebsiella spp. from various clinical conditions in this part of the country is 24.88% as detected by the genotypic method. The study suggests that ESBL production should be detected routinely to monitor the resistant organisms for implementation of appropriate infection control measures.

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17.020

A Novel Extended-Spectrum SHV-Type Beta-lactamase, SHV-104, From Klebsiella pneumoniae

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Background: Since first reported in Germany in 1983 and in the United States in 1989, extended-spectrum β-lactamases (ESBLs) have spread worldwide. These enzymes are mostly plasmid-encoded derivatives of TEM-1, TEM-2, and SHV-1 by one or more base pair changes or are from a rapidly evolving class called CTX-M.

Methods: K. pneumoniae ML2011 was collected on July 2004, from intensive care unit of Military hospital in Tunisia. Identification of strains was performed by using both API 20 E and the VITEK automated system. Minimal inhibitory concentrations (MICs) were determined by E-test Strips for the strain on Mueller-Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI/National Committee for Clinical Laboratory Standards [NCCLS], 2006). Transfer of resistance phenotypes was performed by transformation and conjugation experiments. The ESBL was identified by double-disk synergy test, by isoelectric focusing and sequencing of PCR products.

Results: MICs for K. pneumoniae ML2011 showed that this strain was resistant to all β-lactams tested except imipenem. K. pneumoniae ML20011 exhibited a high level of resistance to oxyiminocephalosporins. The strain was also resistant to kanamycin, chloramphenicol, ciprofloxacin, nalidixic acid, tetracycline and streptomycin.

The disk diffusion method showed synergy between ceftazidime, cefotaxime, aztreonam, ceftriaxone, and amoxicillin-clavulanic acid against the strain and its transformants and transconjugants, suggesting plasmid-mediated production of an ESBL enzyme. PCR analyses confirmed the presence of blaSHV in parent strain K. pneumoniae ML2011, and its transformants E. coli DH5α/pML2011 and transconjugants E. coli HB101 X pML2011 indicating that this gene is located on transferable plasmid with estimated molecular size of 50kb. Nucleotide sequence was performed on the coding region 861pb used to predict the amino acid sequence. This sequence was compared with strain K. pneumoniae Kp297 (DDBJ/EMBL/GeneBank accession no. EF035567) for nucleotide sequence homology and predicted amino sequence. Two amino acid substitutions were found at position 5 and 202, resulting respectively in a Met (ATG) to Leu (TTG) and an Arg (CGT) to Ser (AGT) changes. As these substitutions not showed by other known SHV β-lactamases, the pl 7.3 from K. pneumoniae ML2011 appears to be a novel ESBL and has been designated SHV-104 (http://www.lahey.org).
Conclusion: The modification at position 202 may result in a change of the pl to 7.3 and the extended spectrum of the enzyme.

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17.021

Highly Susceptible Strains of Typhoid Bacilli Encountered in Jamaica

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Background: Unlike reports of multidrug-resistant Salmonella enterica serovar typhi (S. typhi) in countries around the world, strains encountered in Jamaica have been uniformly susceptible to all the anti-typhoid drugs and also to other antibiotics by disc method. We have been maintaining these isolates on Dorset-egg medium over the years. In this report, we examine the MICs of 4 front-line antibiotics against 41 unduplicated isolates (one from a patient) encountered in consecutive 17 years between 1984 and 2000 at the University Hospital in Kingston, Jamaica.

Methods: The MICs were determined by E test (AB Biodisk, Solna, Sweden) using E. coli ATCC 25922 as control. Manufacturers’ instructions in regard to media, inoculum density and incubation parameters were followed strictly. Our observation of extremely low MICs (see results below) made us to do the tests repeatedly and read the results independently by each of us (NCB and OH) and repeat again if we differed in reading by more than one E test dilution.

Results: The MICs (µg/ml) of the four antibiotics were Chloramphenicol MIC range 2-4, MIC50 3 and MIC90 3; Ampicillin MIC range 0.125-1, MIC50 0.25 and MIC90 0.5; Trimethprim/Sulpha MIC range 0.023-0.064, MIC50 0.032 and MIC90 0.047; Ceftriaxone MIC range 0.023-0.047, MIC50 0.032 and MIC90 0.047. All isolates were susceptible. MICs were extremely low, fell in a narrow range and far below the standard susceptible (CLSI) Breakpoint MICs of the antibiotics. We have not seen any report of such a highly susceptible strains of typhoid bacilli from anywhere in the world.

Conclusion: Considering the growing increase of multidrug-resistant typhoid in countries around the world and reports of isolation of strains with MICs of front-line antibiotics of more than 256 µg/ml (Hirose K et al Antimicrob Ag Chemother 45:956-958,2001), the highly susceptible nature of strains encountered in Jamaica is noteworthy. These unique strains which we call ‘Jamaica strains’ have been persisting in this island country throughout the years.

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