

were nosocomial in origin. ESBL in intensive care unit (ICU) and non-ICU patients was 86% and 98% respectively. Among ESBLs in ICU, 13.5% were community acquired. Resistance to LEV was 73.3% > P/T 27.3% and >AMK 12.3%. No resistance to IMP and MER seen. Overall 61% were MDR. Four KS (1.7%) isolates were resistant to ERT. PCR showed blaCTX-M in 73%, blaTEM in 56% and blaSHV in 38%. Multiple genes were present in 60%.

**Conclusion:** Prevalence of ESBL among GNB causing infections continues to be high in Indian medical centers. ERT shows good activity equivalent to the tested Group 2 carbapenems and may be considered for treatment of such infections.

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17.014

#### Early Detection of ESBL Producers from Clinical Samples Using MacConkey Agar with Ceftazidime

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**Keywords:** CMAC; ESBL Lactose-fermenters

**Objectives:** MacConkey agar with ceftazidime (CMAC) was used for early detection of extended spectrum beta-lactamase (ESBL) for clinical sample received from ICU.

**Methods:** A total of 374 clinical samples were received from ICU in the Dept. of Microbiology. Along with MacConkey agar and Blood agar, the samples were processed on MacConkey agar with ceftazidime (1 mg/L) for provisional detection of ESBL isolates. Lactose -fermenting colonies on MacConkey agar with ceftazidime (CMAC) were provisionally detected as ESBL producing isolates. These isolates were identified by standard methods. Presence of ESBL was determined by CLSI method (cefotaxime and ceftazidime disks with and without clavulanic acid).

**Results:** 128 isolates showed growth on CMAC of which 50 were lactose fermenters. 31 isolates were identified as *E.coli* and 19 as *K.pneumoniae*. All the 50 isolates were identified as confirmed ESBL producers.

**Conclusion:** Compared to phenotypic identification of ESBL producers, CMAC helps in early detection ESBLs which can be very useful from treatment point of view. Since in our hospital we have about 60% of ICU patients growing ESBL producers, this early detection helps the intensivist to start anti-ESBL therapy.

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#### Identification of AmpC Beta-Lactamases Using Phenotypic Tests and PCR in Clinical Isolates of *Klebsiella spp.* and *Escherichia coli*

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**Background:** Indiscriminate use of beta-lactam antibiotics has resulted in emergence of AmpC Beta-lactamases. Considering the lack of comprehensive studies from India the present study was undertaken to detect and characterize AmpC Beta-lactamases in clinical isolates of *Escherichia coli* and *Klebsiella* spp.

**Methods:** One hundred non-repeat clinical isolates each of *Escherichia coli* and *Klebsiella* spp recovered from pus, urine, blood and sputum, were collected from various hospitals of Delhi. These were screened for susceptibility to cefoxitin (30 µg) by disc diffusion method. The screen positive isolates were subjected to Modified 3 Dimensional test (M3D), AmpC disk tests 1 and 2 and Inhibitor (Boronic acid) based detection method. AmpC positive isolates were subjected to Polymerase chain reaction (PCR) using family specific primers and also tested for production of ESBLs and inducible AmpC Beta-lactamases.

**Results:** 34/100 (34%) of *Escherichia coli* and 21/100 (21%) of *Klebsiella* spp. were resistant to cefoxitin. Of the screen positive isolates 12 (35%) of *Escherichia coli*, and 5 (24%) of *Klebsiella* spp. were positive for AmpC by various phenotypic tests. Out of the AmpC positive isolates as many as 8/12 of *Escherichia coli* and 3/5 of *Klebsiella* isolates were positive for ESBLs. In 3/12 (25%) of *Escherichia coli* and 3/5 (60%) of *Klebsiella* isolates AmpC were inducible. PCR identified the AmpC of *Escherichia coli* as MIR/ACT-I and CIT types. Amongst *Klebsiella* isolates, the AmpC were found to be of MIR/ACTI, CIT, FOX and DHA families.

**Conclusion:** The incidence of AmpC production was higher (12%) in *Escherichia coli* than in *Klebsiella* (5%) isolates. However, co-production of ESBLs was much higher in *Klebsiella* (60%) than in *Escherichia coli* (25%) isolates. The AmpC Beta-lactamases in Indian isolates were of common families reported from different parts of the world.

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17.016

#### Pooled Analysis of Resistance Patterns of *Escherichia coli* Strains Isolated From Urine Cultures in Turkey: Comparison of 1997–2001 and 2002–2007 Periods

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**Objectives:** Urinary tract infections (UTI) are one of the most common infectious diseases diagnosed in outpatients and also constitutes the most common nosocomial infection in many hospitals. *Escherichia coli* remains the principal causative pathogen of UTIs both in outpatients and inpatients. In this study it was aimed to compare the resistance patterns of *E. coli* strains reported to be isolated from urine

cultures in published medical literature from Turkey in the 1997–2001 and 2002–September 2007 periods.

**Method:** To find out the published series three national databases (Ulakbim Turkish Medical Literature database, <http://www.turkishmedline.com>, <http://medline.pleksus.com.tr>) and two international databases (Pubmed and Science Citation Index (SCI)) were searched. Keywords for national databases were ["idrar yolu infeksiyonu" or "idrar yolu enfeksiyonu" or "urinary tract infection" or "üriner sistem infeksiyonu" or "üriner sistem enfeksiyonu"]. Keywords for Index Medicus and SCI-expanded were ["urinary tract infection" and Turkey]. Articles i)published before 1997 ii)resistance data of outpatient and inpatient strains were not analysed separately iii)antibiotic susceptibility data were not given, were excluded. All studies used Kirby-Bauer disc diffusion test by using NCCLS/CLSI criteria for determination of antimicrobial resistance. Resistance data of inpatient and outpatient strains were compared by Chi-square test. A *p* value less than 0.05 was considered significant.

**Results:** Data for 25577 *E. coli* strains were obtained from 53 articles (28 articles from 1997–2001 period, 25 from 2002–2007 period). Of these strains 18106 were isolated from outpatients whereas 7471 from inpatients. The resistance rates and comparisons of 1997–2001 and 2002–2007 periods are shown in Table 1.

**Conclusions:** Trimethoprim sulphamethoxazole resistance is very high and it cannot be recommended in the empirical treatment any more. Nitrofurantoin may be a cheap and reasonable option in uncomplicated UTI. Aminoglycosides and third-generation cephalosporins may be good choices in the treatment of complicated UTI. Carbapenems may be conserved for extended-spectrum beta lactamase producing strains. ESBL rate in the outpatient strains is alarming. Policies to constrain resistance such as antibiotic stewardship or restriction programmes should be implemented immediately.

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#### 17.017

### OXA- and MBL-Type Enzymes Among Uncommonly Isolated *Acinetobacter* Spp. in Asia-Pacific Nations: Natural Reservoir for Resistance Determinants

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**Background:** *Acinetobacter* (AC) other than *A. baumannii* (ACOB) are occasionally recovered from clinical sources, but OXA and MBL genes have rarely been reported. The aim of this study was to identify and characterize OXA and MBL genes in AC. Gene context of *bla*<sub>OXA-23</sub> detected in *A. radiosistans* (AR) was also evaluated.

**Methods:** AC recovered from patients in 10 countries in the Asia-Pacific (APAC) region were tested by broth microdilution. Isolates with imipenem or meropenem MIC  $\geq 8$  mg/L were screened for MBL- (IMP-like, VIM-like, SIM-1, GIM-1, SPM-1) and OXA- (OXA-23, -24, -58 clusters) genes. OXA genes and surrounding sequences were assessed by PCR

using primers targeting to *ISAbA1*, 2 or 3, or degenerate primer approach. Southern blot and hybridization of chromosomal and plasmid DNA were performed. RNA extraction followed by reverse transcriptase-PCR was performed to access expression of *bla*<sub>OXA-23</sub>. Species identification was confirmed by 16S rRNA.

**Results:** Among 543 AC isolates, 28.2% carried OXA (98.7%) or MBL (1.3%) genes, from which 2.6% were found ACOB. An *A. junii* (AJ) with *bla*<sub>OXA-23</sub> and an AR with *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> were detected in 2 Indian centers, while an *A. johnsonii* with *bla*<sub>IMP-4</sub> and *A. calcoaceticus* (ACA) with *bla*<sub>OXA-58</sub> were identified in the Philippines and China, respectively. *ISAbA1* and *ISAbA3* surrounded *bla*<sub>OXA-23</sub> from AJ and *bla*<sub>OXA-58</sub> from AR and ACA, respectively. The AR showed a putative O-sialoglycoprotein endopeptidase-encoding gene upstream of *bla*<sub>OXA-23</sub>; gene expression was not detected; and *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-58</sub> were located on the chromosome and plasmid, respectively. Susceptibility to polymyxin and tigecycline ( $\leq 2$  mg/L; no breakpoint criteria) was  $\geq 98.9\%$ .

**Conclusion:** High dissemination of OXA genes was detected, emphasizing their ability to spread among AC. The data suggest AR may be a natural reservoir for *bla*<sub>OXA-23</sub>. *bla*<sub>IMP-4</sub> has been previously detected in Hong Kong, Australia and now Philippines, highlighting spread in APAC.

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#### 17.018

### Mutation in DNA Gyrase and Topoisomerase IV of *V. cholerae* Causing Diminished Susceptibility to Ciprofloxacin

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**Background:** Diminishing clinical response to ciprofloxacin (CIP) therapy at ICDDR, B correlated with decreased minimum inhibitory concentration (MIC) of *V. cholerae* to CIP, though strains remained susceptible using standard thresholds. To determine the cause of the diminished susceptibility, we investigated mutations in the quinolone-resistance-determining-region/analogous region of genes *gyrA/B* and *parC/E* that encode, respectively, quinolone targets DNA gyrase and topoisomerase IV.

**Method:** We studied 30 clinical isolates of *V. cholerae* O1 and 10 of *V. cholerae* O139 isolated during 1993–2006. Susceptibility was determined by standard disk diffusion (DD) and E-test methods and interpreted according to CLSI and manufacturer's instruction respectively. We extracted chromosomal DNA, amplified and sequenced the gene fragments and then edited, matched and aligned the sequences using suitable methods and software to identify mutations.

**Results:** No mutation was detected in 9 strains having a CIP MIC of 0.002–0.003 and a NA MIC of 0.125–0.5  $\mu$ g/ml; a substitution (Aspartic acid 87Asparagine) was noted in *gyrA* was noted in 2 strains with a CIP MIC of 0.008–0.012 and a NA MIC of 4–8  $\mu$ g/ml; a unique *gyrA* mutation in