nor to a susceptible K. pneumoniae from a patient in a geographically separate hospital. PCR product of the expected sizes were obtained from both strains using primers specific for  $bla_{\text{TEM}}$ ,  $bla_{\text{SHV}}$ ,  $bla_{\text{GES}}$  related genes. Additionally,  $bla_{\text{CTX-M}}$  was detected in strain B. Analysis of the DNA sequencing data of the  $bla_{\text{GES}}$  product from strain A shows that it is similar to  $bla_{\text{GES-2}}$  and  $bla_{\text{GES-3}}$ , sequences. Conclusions: It is possible that GES accounts for carbapenem resistance in strain A, which is cause for concern. Genes encoding these enzymes have been identified on integrons, which facilitates their spread.

## C2-2065 Characterization and Sequence Analysis of Extended Spectrum β-Lactamase Encoding Genes from E. coli, K. pneumoniae and P. mirabilis Isolates Collected During Tigecycline (TGC) Phase 3 Clinical Trials.

M. TUCKMAN, D. KEENEY, R. CAMARDA, S. MANDIYAN, T. SANDS, A. V. RUZIN, P. A. BRADFORD, C. H. JONES; Wyeth, Pearl River, NY.

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Background: In concert with the development of novel \( \beta\)-lactams and broad-spectrum cephalosporins, B-lactamases have evolved to accommodate the new agents. This study was designed to identify, at the sequence level, the genes responsible for the ESBL phenotype of E. coli, K. pneumoniae and P. mirabilis isolates collected during the global TGC phase 3 clinical trials. Methods: Bacterial isolates were collected from patients enrolled in phase 3 clinical trials for TGC. Antibiotic susceptibility was determined by broth microdilution as recommended by the CLSI. The ESBL phenotype was confirmed using Etest® ESBL strips for both ceftazidime (CAZ) and cefotaxime. PCR assays were developed to identify and clone the  $bla_{\text{TEM}}$  $bla_{\text{SHV}}$   $bla_{\text{OXA}}$ , and  $bla_{\text{CTX}}$  determinants from clinical strains. Isolates were also screened for AmpC genes and the  $bla_{\text{KPC}}$  genes. **Results:** *E. coli, K. pneumoniae* and *P. mirabilis* isolates with a CAZ MIC  $\geq 2~\mu\text{g/mL}$  were designated as possible ESBL positive pathogens. Those isolates that were either ESBL positive or indeterminate by Etest were analyzed further by PCR for characterization of encoded \( \beta \)-lactamases. A total of 2123 isolates representing 1330 patients were screened. 117 of these were confirmed to encode at least one ESBL, with 45% of positive isolates encoding multiple ESBLs. 90% and 75% of ESBL positive E. coli and K. pneumoniae isolates. respectively, encoded a CTX-M-type β-lactamase gene; whereas, 67% and 28% of K. pneumoniae and E. coli ESBL positive isolates, respectively, encoded an SHV gene. TGC susceptibility was unaffected by the presence of an ESBL with 94% of the isolates susceptible to TGC (MIC<sub>50</sub> = 2  $\mu$ g/ mL). Conclusions: This study identified the genetic determinant(s) of ESBL mediated CAZ resistance in 117 unique patient isolates from the TGC phase 3 clinical trials and demonstrated that TGC may have therapeutic utility against strains producing ESBLs.

## C2-2066 Epidemiology of Multi-Drug Resistant Escherichia coli in England, 2002 to 2006.

M. LHLLIE, A. PEARSON, A. JOHNSON; Hith. Protection Agency, London, United Kingdom.

Background: Several European studies have reported increased prevalence of Escherichia coli resistant to extended-spectrum cephalosporins and fluoroquinolones. We investigated the incidence of multi-drug resistant E. coli in England from 2002 to 2006. Methods: Isolate susceptibility data were abstracted from the Health Protection Agency's national database (LabBase2) to which hospital microbiology laboratories across England routinely report, on a voluntary basis, clinically significant cases of bacteremia. Only isolates with susceptibility data for extended-spectrum cephalosporins, ciprofloxacin and gentamicin were included in analyses. Results: A total of 46,257 isolates with susceptibility data for all three antibiotics were collected in England from 2002 to 2006. The percentage of isolates susceptible to all three antibiotics has decreased from 88.2% in 2002 to 73.7% in 2006. The percentage of isolates resistant to all three antibiotics increased fourfold from 1.2% in 2002 to 5.0% in 2006 (Table 1).

Table	<ol> <li>Multi-drug resistanto 2006</li> </ol>	ce (percent) in E. col	blood isolates rep	ported in England,
Year	Isolates resistant to extended-spectrum cephalosporins	Isolates resistant to ciprofloxacin	to gentamicin	Isolates resistant to all three antibiotics
2002	2.8	8.9	5.3	1.2
2003	4.8	13.6	7.2	2.7
2004	6.8	16.5	8.1	3.2
2005	9.6	20.0	8.7	4.0
2006	12.1	23.1	9.6	5.0

Voluntary reporting for the presence of ESBLs did not begin until 2006. Of 37 extended-spectrum cephalosporin resistant isolates, 31 were reported as ESBL producers. **Conclusions:** These data show significantly increased rates of multi-drug resistant *E. coli* among blood isolates reported in England from 2002 to 2006.

## C2-2067 Aquatic Environmental Contamination with ESBL-Producing *Enterobacteriaceae*.

E. MACHADO<sup>1, 2, 3</sup>, T. M. COQUE<sup>3</sup>, J. C. SOUSA<sup>1</sup>, D. SILVA<sup>2</sup>, M. RAMOS<sup>2</sup>, J. ROCHA<sup>2</sup>, H. FERREIRA<sup>2</sup>, L. PEIXE<sup>2</sup>; <sup>1</sup>UFP, Porto, Portugal, <sup>2</sup>Requimte, FFUP, Porto, Portugal, <sup>3</sup>HU Ramón y Cajal, Madrid, Spain.

Background: The occurrence of ESBL- and other beta-lactamase-producing organisms from aquatic environments and their relationship with those recovered in hospitals have been scarcely analyzed. Methods: ESBLproducing Enterobacteriaceae (n=16) were detected in urban wastewater downstream 4 hospitals (n=12), river (n=2), and sea waters (n=2) in Porto area, Portugal (2001-04). Samples were plated on MacConkey agar with and without 1 mg/L ceftazidime or cefotaxime after filtration. Bacterial identification, antibiotic susceptibility and transferability were determined by standard methods. ESBL characterization included synergy test and identification of known bla genes (PCR, sequencing). Clonal relatedness was established by RAPD-PCR. Class 1 and class 2 integrons were identified by PCR, RFLP-typing and sequencing. Results: ESBL-Enterobacteriaceae were recovered from hospital sewage (TEM-10, -52, -116; SHV-2, -27), river (TEM-24, -52) and sea waters (CTX-M-14, -32). They were mostly resistant to aminoglycosides, quinolones, trimethoprim, sulfonamides, tetracyclines and chloramphenicol. The hospital disseminated TEM-24-producing E. aerogenes clone was observed. Transferable plasmids of ca. 100kb and 40kb, harbouring  $bla_{\text{CTX.M-I4}}$ , or  $bla_{\text{CTX.M-32}}$ , respectively, were detected. Three class 1 integrons were identified: aacA4 (associated with TEM-24); aadA2 (in a TEM-116- K. pneumoniae), and aacA7-bla<sub>VIM-2</sub> (in a K. pneumoniae also harbouring TEM-10 and SHV-27 and co-transferable with ESBL genes). Two class 2 integron-types were identified: dfrA1-sat2-aadA1-orfX and the new dfrA1-sat2. Conclusions: Detection of multiresistant ESBL- and VIMproducing isolates, integrons, and clonal relationship with epidemic clones disseminated in the nosocomial setting, suggest environmental contamination from hospitals. Control of release of these bacteria should be a public health priority in our country.

## C2-2068 Effective Risk Factors for Colonization of Extended-Spectrum β-Lactamase Producing Gram-Negative Bacilli in Hospitalized Neonates.

R. MONIRI, Z. MOSAYEBI, G. MUSAVI; Kashan Univ. of Med. Sci., Kashan, Iran (Islamic Republic of).

Background: The occurrence of extended-spectrum β-lactamase (ESBL) producing isolates has increased worldwide. The purpose of this study was to prospectively determine the rate of beta-lactam antibiotic resistance in commensal fecal flora of newborns and the risk factors lead to their colonization. Methods: Total of 167 hospitalized newborns were enrolled. The ESBL production was investigated on isolates by double-disk synergy test. Results: Colonization with gram negative commensal fecal flora microorganisms was determined in 120 stool samples. Klebsiella pneumonia and Escherichia coli were identified in 53 (44.2%) and 34 (28.3%) of the samples, respectively. Microorganisms producing ESBL were identified in 36 (29.2%) stool samples. 23 of 35 (65.7%) microorganisms producing ESBL were K.pneumoniae. The frequencies of co-resistance in ESBLs isolates were as follows: ciprofloxacin 5.7%; imipenem 17.1%; meropenem 91.4%; clindamicin 97.1%; carbenicillin, 100%; and ticarcillin 100%. The main risk Factors for Colonization of ESBL producing gram negative