

isoelectric focusing analysis, polymerase chain reaction and sequencing, we detected the major genotypic characterization of ESBLs was CTX-M-14 (76.2%). Two strains showed indistinguishable patterns by pulsed-field gel electrophoresis.

Conclusion: This study documented the CTX-M family as the predominant ESBL type among Macao population. The spread of CTX-M enzymes is concerning and deserves close monitoring in further investigation.

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23.017

Utilizing hospital generated antibiograms to examine state trends in antibiotic resistance

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Background: Antibiograms are aggregated, hospital-generated reports on susceptibility of bacteria of interest to specific antibiotics. They are utilized within hospitals to assist in effective use of antibiotics. The Massachusetts Department of Public Health (MDPH) has been requesting voluntary submission of antibiograms from hospitals annually since 1999.

Methods: Susceptible proportions reported in antibiograms were analyzed to evaluate changes in levels of susceptibility over five years, while accounting for the effect of hospital characteristics. Trends were examined for specific antibiotic and bacteria combinations as well as antibiotic class susceptibility patterns. Data were analyzed using SAS software version 9.1 (SAS Institute Inc., USA).

Results: Significant trends in antibiotic resistance were seen with a strong decreasing trend in *E.coli* fluoroquinolone-susceptibility and a moderate decrease in *Klebsiella pneumoniae* and *Enterobacter cloacae*. Specifically, *E.coli* susceptibility to ciprofloxacin decreased substantially over five years, and this trend was more pronounced in specific regions of the state. Other hospital characteristics such as bed count and hospital type did not appear to have a significant association with antibiotic resistance trends.

Conclusion: Antibiograms may serve as useful tools in examining regional antibiotic resistance trends. Trends identified may be used to inform further studies and pinpoint areas of concern for hospitals.

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A Comparative study on gram-negative bacterial infections in Mansoura University Hospitals, Egypt

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Background: Gram negative bacteria are responsible for numerous infectious diseases. These diseases can occur in and harm any part of the body, the skin, eyes and the

nervous, cardiovascular, respiratory, gastrointestinal and urogenital systems.

Methods: In the present study, some phenotypic and molecular typing techniques were applied on 108 strains of *E. coli*, 88 strains of *Ps. aeruginosa* and 8 strains of *Serratia* isolated from different clinical lesions in Mansoura University Hospitals, Egypt.

Results: The distribution of antibiotic resistance among the isolated strains showed high incidence of resistance and imipenem was the most active antibiotic. Using the active pyocin typing, 72 strains of *Ps. aeruginosa* could be typed into 35 pyotypes. SDS-PAGE of total cell protein extracts showed that the presence of fifteen patterns among *E. coli* strains and eleven patterns among *Ps. aeruginosa* strains. Using PCR technique it was found that 84% of the 50 tested strains were found to have at least one of the tested ESBLs. Also *TolC* and *AcrA* genes were present in all tested *E. coli* except 4 strains and did not present in *Ps. aeruginosa* except 4 strains. Plasmid profiles of 23 tested *E. coli* appear to be diverse. Also the prevalence of plasmids in 22 tested *Ps. aeruginosa* strains was lower than in tested *E. coli* therefore 59.1% of tested *Ps. aeruginosa* strains harbored plasmids. Using Pyrosequencing technique, the sequenced region of *gyrA*, *gyrB* and *ParC* were able to differentiate between the tested strains and neighbor-joining tree was constructed to determine relatedness between the isolated strains. Moreover, Molecular cloning of the whole sequence of *bla-TEM*, *bla-SHV* and *bla-CTX-M* was carried out experimentally to study the expression of these genes and determine which genes of them responsible for the resistance.

Conclusion: Molecular-based methods of typing are more advantageous compared with phenotypic methods of typing in terms of better discrimination and reproducibility. Significant genetic variation was observed among different strains represented by the diversity of their plasmid profiles. All molecular genetic methods for distinguishing organism subtypes are based on differences in the DNA sequence.

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Extended spectrum beta-lactamases in *Escherichia coli* and *Klebsiella* spp. from Eastern Romania

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Background: The emergence and dissemination of ESBL are problems of major importance for the population health; ESBLs represent a first example of factor that contribute to the global crisis concerning the treatment of *Klebsiella pneumoniae* and *Escherichia coli* against which the third generation cephalosporins are not effective anymore.

Methods: Clinically isolates of *E. coli* (n= 642) and *Klebsiella* (n=92) were collected from patients with different types of infections (sepsis, urinary tract infections,

etc), hospitalized between september 08 and september 09 in a Hospital of Infectious Diseases from Eastern Romania. Double disc synergy test using cefotaxime and amoxicillin/clavulanic acid discs was used to screen ESBL producers and these strains were subsequently subjected to confirmatory Etest.

Results: *E. coli* resistance to cefotaxime, ceftazidime, cefoxitin, ciprofloxacin and imipenem was found to be 40, 29, 6, 30 and 1% respectively. *Klebsiella* resistance to cefotaxime, ceftazidime, cefoxitin, ciprofloxacin and imipenem was 70, 57, 22, 41 and 4% respectively. % of ESBL producers *E. coli* was 15% (97 strains) and *Klebsiella* was 38% (35). All the ESBL producing strains were susceptible in 100% to imipenem and meropenem.

Conclusion: Carbapenems remain the most active agents against Gram-negative isolates, including ESBL producers strain of *E. coli* and *Klebsiella* spp. isolated from community-acquired and nosocomial infections from Eastern Romania.

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Can we rely on automated VITEK2 system the detection of KPC and other class A carbapenemase producers enterobacteriaceae?

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Background: Class A carbapenemases have become more prevalent within Enterobacteriaceae. Proficient methods are needed for their early detection in clinical microbiology laboratories in any attempt aimed for targeting optimal antimicrobial therapy and controlling their spread. Automated systems, as VITEK2, are increasingly used for routine susceptibility testing to decrease the in-laboratory turnaround time. However, the performance of VITEK2 for the whole class A carbapenemase family detection has never been assessed before. Objective: to determine the performance of VITEK2 for carbapenemase detection compared with both, CLSI agar dilution MIC and the genotype obtained by molecular methods.

Methods: Methods: we designed a panel composed by diverse bacterial genera with distinct carbapenem susceptibility patterns composed by 37 carbapenemase producers and 34 nonproducers (n): KPCs (17) Sme (10), NMC-A/IMI (2), GES (4), VIM/IMP (4) and CTX-M (12), AmpCs (12), combined mechanisms and others (10), respectively. The resistance mechanisms of the strains were assessed by PCR/DNA sequencing. Each isolate was tested with the VITEK2 using the AST-N082 cards specifically designed for South American countries (which included only imipenem -IPM- and meropenem -MEM-), according to the manufacturer's instructions and by CLSI agar dilution MICs for both carbapenems. Discrepant results were resolved by retesting the isolates.

Results: Overall categorical interpretations with VITEK2 showed a 72% and 79% of agreement with reference MICs for IPM and MEM, respectively. Very major (VM), major (MA) and minor (MI) errors were: IPM, VM 3%, MA 11% and MI 13%; MEM, VM 4%, MA 6% and MI 11%. Most of the errors (>80%)

occurred among carbapenemase producers. The expert system showed sensitivity (SN) of 76% and specificity (SP) of 87% for carbapenemase detection, when confronted to the genotype, with the greatest SN for KPCs (82%) and the lower for MBLs (25%). The recognition of suspected carbapenemase producers could be increased with the combined use of IPM and MEM with modified cut-off points of ≥ 2.0 mg/L and ≥ 1.0 mg/L, respectively (SN 97%, SP 90%).

Conclusion: VITEK2 may be suitable in clinical laboratories for Class A carbapenemase detection, but should be accompanied with modifications in the cut-off used for screening of suspected carbapenemase producers to ensure their proper detection.

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23.021

Emergence of multidrugresistant gram negative bacilli and enterococci from rectal swabs of newborn and their mothers from Central India

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Background: Newborn babies acquire gut flora mainly from mother and surrounding. We had observed in the faecal samples of newborns prevalence, colonization of multidrug resistant, ESBL pandrug resistant gram negative bacteria, vancomycin resistant enterococci as a new threat in the newborn admitted in hospital. The influx of these bacteria into hospitals has major implications for infection-control and empirical treatment strategies.

Methods: A total of 140 samples of faeces from neonates and mothers admitted in general maternity ward and ICU of two hospital in central India were examined within 24-48 hours for presence of ESBL, pandrug resistant gram negative bacilli, and vancomycin resistant enterococci. Antibiotic susceptibility test were performed using Kirby-Bauer disc diffusion method and results were interpreted according to CLSI. Van A and ESBL gene was confirmed using E test, PCR and RT PCR.

Results: 1. A total of 48 *E. coli*, 49 *Klebsiella*, 21 *Pseudomonas* and 52 *Enterococci* isolates were obtained. The percentage of multidrug resistant *E. coli*, *Klebsiella*, *Pseudomonas* and *enterococci* was 78.79, 66.67, 58.8 and 91.18% respectively. For *gram negative bacilli* % resistance for chloramphenicol (47%), carbapenem 58.9% and ampicillin (74.3%) aminoglycosides (70.9%), quinolones (65.8%) and cefoparzone+ sulbactam (58.1%), piperacillin+tazobactam (69.2%) cotrimoxazole (47%) cephalosporin (71.1%). The prevalence of ESBL gene (TEM and SHV) among *E. coli* and *Klebsiella* was 100% and 75% respectively. The pandrug resistant was 18.15% among *E. coli* and 20.4% among *Klebsiella* and 1.5% among *Pseudomonas* Of 52 enterococci, 47.06% of them were vancomycin resistant strain and harboured van A gene. Enterococci were showing a high level resistance to aminoglycosides (82.35%), ampicillin (82.35%), chloramphenicol (38.24%), teicoplanin (44.12%) and linezolid (8.82%).

Conclusion: We report high rates of colonization with ESBL and pandrug resistant gram negative organism and