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Abstract of Thesis Paper

Title: The growing problem of antibiotic resistance in clinically and hospital environment relevant Gram–negative bacteria: current situation in Tunisia

Antimicrobial resistance is a major health problem worldwide, but marked variations in the resistance profiles of bacterial pathogens are found between countries and in different patient settings. In Tunisia, the strikingly high prevalence of resistance of bacteria to penicillins and cephalorosporins drugs including fourth generation in clinical isolates of Gram negative bacteria has been reported. During 30 years, the emerging problem of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates is substantial, and some unique enzymes have been found. Recently, evidence that Gram-negative bacteria are resistant to nearly all available antimicrobial agents, including carbapenems, have emerged.

We investigate in this study extended-spectrum β-lactamases (ESBL)-producing *Enterobacteriaceae* in the hospital environment. Swabs were collected from ten different area from Kasserine hospital, Tunisia. Totally of 46 strains were subcultured onto MacConkey agar supplemented with ceftazidime for the detection of ESBL-producing *Enterobacteriaceae*. Identification and susceptibility pattern were done using Phoenix automated phenotypic identification criteria. Extended spectrum β-lactamases (ESBLs) were detected using cefepime ESBL Etest. Colony blotting was first used to detect the presence of bla_{SHV}, bla_{CTX-M}, bla_{CMY}, bla_{IMP} and bla_{VIM} genes. PCR was used to amplify these genes and the amplicons were sequenced and analysed. Total DNA was digested with Spe1 and PFGE was used to type the major isolates. Among the 46 strains, 63% are *Klebsiella pneumoniae*, 13% are *Escherichia coli*, 8.7% are *Proteus mirabilis*, 6% are *Enterobacter cloaceae*, 4.3% are *Providencia rettegri*, 2.5% are *Serratia marcescens* and 2.5% are *Pantoea agglomerans*. Hospital environment isolates produces SHV-125, CTX-M-15, CMY-2 ESBLs and IMP-1 and VIM-2 MBLs. Typing by PFGE showed that of IMP-1 metallo-β-lactamase producing *K. pneumoniae* isolates were not genetically identical, and are not clonal.

Enterococcus faecalis is an emerging nosocomial pathogen that can cause difficult-to-treat infections and exhibits significant degrees of poorly understood multi-drug resistance

mechanism. *E. faecalis* is thought to possess a great deal of intrinsic resistance to several antimicrobial agents, including chloramphenicol, although the mechanisms involved in such resistance are not well understood. In this work we found ampicillin- and erythromycin-resistant clinical isolates of *E. faecalis* with MICs >16 μg/ml. PCR amplification showed that the twelve resistant isolates in the current study produced TEM-1-like β-lactamase, OMP preparation and protein sequencing were used to study the distribution of *mefA* and *msrA* efflux pumps in these twelve epidemiologically distinct groups of Enterococci. These determinants were responsible for erythromycin resistance in over these twelve of clinical isolates of *E. faecalis*, these efflux pumps are closely related to the major ABC and MFS efflux pump families. These determinants have previously only been found in a small number of epidemiologically related strains of *E. faecalis.*