Prevalence of extended-spectrum-ß-lactamase (ESBL) and metallo-ß-lactamase (MBL) antibiotic resistance genes in *Enterobacter* species in Pretoria Academic Hospital

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Introduction

- *Enterobacter* spp are opportunistic pathogens that rarely colonise healthy humans
- The pathogenic *Enterobacter* spp harbour extended-spectrum β-lactamase (ESBL) and metallo-ß-lactamase (MBL) enzymes
- The ESBL enzymes were first identified in 1980 in Germany and are frequently the products of three genes bla_{CTX-M}, bla_{SHV} and bla_{TEM}
- The MBL enzymes were first reported from a *Bacillus cereus* strain in 1999 in Italy. The bacteria harboured the VIM and IMP genes
- Multi-drug resistance due to the presence of the ESBL and MBL resistance genes in *Enterobacteriaceae* is the cause of therapy failure during treatment with 3rd generation cephalosporins and carbapenems
- Detection of ESBL and MBL producing bacteria is based on phenotypic and genotypic techniques
- A multiplex PCR can be used for the simultaneous detection of these resistance genes

Aim

- To investigate the prevalence of ESBL and MBL antibiotic resistance genes in *Enterobacter* species in Pretoria Academic Hospital using a multiplex PCR assay

Materials and Methods

- Ninety-seven (97) consecutive clinical *Enterobacter* isolates were collected from the diagnostic division of the Department of Medical Microbiology
- The study included 16 *E. aerogenes* and 81 *E. cloacae* isolates
- Identification and antibiotic resistance was determined using the Vitek 2 (bioMérieux, France)
- The MagNA Pure Compact (Roche Applied Science, Germany) was used for the extraction of total DNA according to the manufacturer's protocol
- The multiplex PCR amplification assay was performed using the QIAGEN Multiplex PCR kit (QIAGEN, USA) following the manufacturer's instructions on a PX2 Thermal Cycler (Thermo Electron Corporation, MA, USA)

References

- Pitout JD and Laupland KB (2008) Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. *Lancet Infectious Diseases* **8**:159-166
- Sanders WEJR and Sanders CC (1997) *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. *Clinical Microbiology Reviews* **10**:220-241
- Schlesinger J, Navon-Venezia S, Chmelnitsky I, Hammer-Münz O, Leavitt A, Gold HS, Schwaber MJ and Carmeli Y (2005) Extended-spectrum beta-lactamases among *Enterobacter* isolates obtained in Tel Aviv, Israel. *Antimicrobial Agents and Chemotherapy* **49**:1150-1156
- Schwaber MJ, Raney PM, Rasheed JK, Biddle JW, Williams P, McGowan JE and Tenover FC (2004) Utility of NCCLS guidelines for identifying extended-spectrum beta-lactamases in non-*Escherichia coli* and non-*Klebsiella* spp. of *Enterobacteriaceae*. *Journal of Clinical Microbiology* **42**:294-298

Results and Discussion

- The multiplex PCR successfully detected the presence of the bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes in the *Enterobacter* spp isolates from the Pretoria Academic Hospital (Figure 1)
- In total, the prevalence of ESBL antibiotic resistance genes in *Enterobacter* species was 56% (54/97) in this clinical setting
- ESBL genes were detected in 25% (4/16) of *E. aerogenes* isolates, while 75% (12/16) were negative (Figure 2)
- In *E. cloacae*, 62% (50/81) of the isolates harboured ESBL genes, while 38% (31/81) were negative (Figure 3)
- None of the *Enterobacter* isolates analysed in this study were positive for the VIM gene
- A 22% ESBL prevalence in *Enterobacter* spp was reported in the Tel Aviv Medical Centre (Israel) in 2005 (Schlesinger *et al*., 2005), while a much lower ESBL prevalence of 2% was reported in the United States (Schwaber *et al*., 2004)

Figure 1: Gel electrophoresis analysis of *Enterobacter* spp isolates (Lanes 1-14) that were ethidium bromide stained. This multiplex PCR simultaneously amplified the bla_{CTX-M} (593bp), bla_{TEM} (445bp) & bla_{SHV} (747bp). In lane C a negative control was included and in lanes M a 100bp DNA ladder (Promega, Madison, USA) were included. Lanes 2, 3, 5 & 12 are bla_{SHV} and bla_{TEM} positive isolates. In lanes 7, 10, 11 & 13 bla_{CTX-M} & bla_{TEM} positive isolates can be distinguished. Lanes 8 & 14 are *Enterobacter* spp isolates positive for all three ESBL genes analysed. In Lane 9 a *Enterobacter* spp isolate positive for bla_{TEM} can be seen. The isolates in lanes 1, 4 & 6 are negative for all ESBL genes

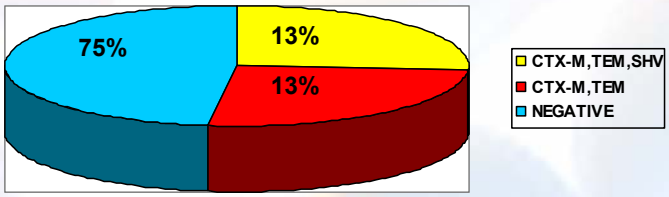

Figure 2: Pie chart representation of the prevalence of the ESBL genes in *E. aerogenes* clinical isolates

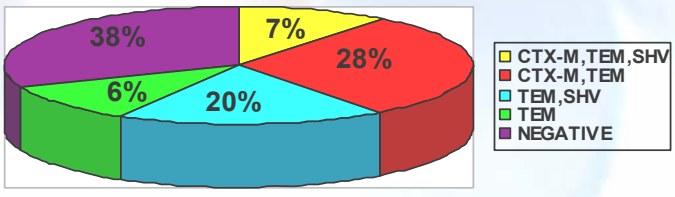

Figure 3: Pie chart representation of the ESBL genes' prevalence in the *E. cloacae* clinical isolates

Conclusion

- The results of this study showed a high prevalence of 56% ESBL genes in *Enterobacter* spp in Pretoria Academic Hospital
- It is essential to include molecular techniques as part of surveillance to monitor the circulation of these resistant genes in a clinical setting