CHAPTER 7: GENERAL DISCUSSION AND FINAL CONCLUSIONS.

"Reviews on extended spectrum beta-lactamases (ESBLs) are numerous, and cynics argue that there is nothing left besides cataloguing minor variants." (David M. Livermore – 3rd International Symposium on Antimicrobial Agents and Resistance, Seoul, Korea, 2001). This view cannot be further from the truth, as rapid development occurs in the bacterial genetic environment, bringing with it an accumulation of enzymes unrelated to the "traditional" TEM- and SHV-type beta-lactamases. Another problematic aspect is the emergence of integron-borne beta-lactamases with their respective armamentariums of co-resistance factors (1, Chapter 3). Recent developments in the class A beta-lactamases of *Pseudomonas aeruginosa* demonstrated exactly this phenomenon with great clarity (2, 3, Chapter 2).

A novel extended-spectrum beta-lactamase enzyme was discovered in the hospital-associated pathogen, *P. aeruginosa* in the PAH during May 2000, in Pretoria, South Africa (4). After chemical and molecular characterization of the enzyme was completed, it became clear that beta-lactamase GES-2 (Guiana Extended-Spectrum) was unique in its composition and antibiotic substrate profile (4), making it the first identification of this specific bacterial enzyme worldwide. What made this enzyme so different from all other known ESBLs was the fact that it was able to confer partial resistance towards the broad-spectrum beta-lactam antibiotic, imipenem (4). To compound matters, the detection of this resistance threat in the routine clinical microbiology laboratory is notoriously slow and laborious. False negative test results, utilizing routine detection methods are fairly common, making diagnosis and infection control extremely difficult (5). Phase 1 research (Chapters 4 and 5) conducted during this study, focused on improving the molecular laboratory detection of bla_{GES-2}. This research produced two highly specific molecular methods of detection. Firstly, the

development of a novel screening technique for this specific resistance mechanism (*bla*$_{GES-2}$) utilizing a peptide nucleic acid-mediated, competitive multiplex PCR method (6, Chapter 4), opened this specific research field to cost effective epidemiological surveys on a national scale. At the time of writing, the PNA-mediated PCR method (6) is already in use to detect *bla*$_{GES-2}$ from *P. aeruginosa* isolates obtained from several South African laboratories (G. Weldhagen – unpublished data). This method, being the first of its kind in the field of ESBL research, certainly through personal adaptation, has the ability to be used in the detection of a variety of very specific ESBL encoding genes. To illustrate the current impetus of PNA in the field of infectious disease research, a similar method targeting point mutations in *rpoB* genes of *Mycobacterium tuberculosis,* was published shortly (1 month) after the ESBL detection work (6), by a Japanese research group (7).

Secondly, to improve the rapid identification and diagnosis of this important resistance threat (GES-2) in the clinical microbiology laboratory, a highly specific and rapid detection process utilizing real-time PCR methodology was developed (8, Chapter 5). In ESBL research, only once before has the process of real-time PCR been used to detect ESBL genes of the SHV family by a German research group (9). Previous work proved that the LightCycler could be used to detect ESBL genes from *Enterobacteriaceae* with great sensitivity and specificity (9). Although still relatively novel in ESBL research, the application of real-time PCR may possibly yield the best results when applied to the division of large ESBL families into genetically similar groups (9). The clinical benefits of such an approach is however still largely uncertain. The sensitive LightCycler method in this study detected *bla*$_{GES-1}$ from one clinical isolate (1 % of isolates tested) (8), proving once and for all that both *bla*$_{GES-1}$ and *bla*$_{GES-2}$ circulate in South African isolates of *P. aeruginosa*. GES-2 however,

with a broader substrate profile, seems to be more prevalent in, and advantageous to, this species. This finding correlates well with those of other studies where *bla*$_{GES-1}$ occurred only in isolated specimens of *P. aeruginosa* (10, 11). Recent reports detailing novel point mutations in *bla*$_{GES}$ genes, is typically conspicuous in the absence of *P. aeruginosa* isolates either carrying or producing these genetic structures (12, 13, 14). Although too early to come to a final conclusion, the relatively low (51.5%) G+C-content of GES-type genes (4) and the propensity of *P. aeruginosa* genes to utilise cytosine and guanine in the wobble position (15) may possibly explain the incompatibility of these genetic structures with this species. Although highly sensitive and specific, the detection methods described in this thesis however, do not give one any insight into areas outside of the chosen target site and are therefore unsuitable to detect new GES-type variants without prior adaptation.

Phase 2 research of this thesis (Chapter 6), focused on determining the genetic stability of *bla*$_{GES}$–type genes in their integron-borne genetic environment, whilst under selective antibiotic pressure. Results obtained, showed that short-term exposure of GES-type-producing organisms to sub-inhibitory concentrations of single antimicrobial agents, are unlikely to select significant spontaneous mutations in these beta-lactamase genes. In addition to these findings, the regulatory mechanisms of *bla*$_{GES}$-type genes remained genetically stable during the experiment. Although these are in essence negative findings, it may still add some value to the research process and possibly offer the clinician some degree of reprieve from the short-term abuse of antibiotics. The GES family of ESBLs is still in its infancy and under no circumstances should one assume that evolution and dissemination of these genetic structures would slow down in any way. That is, unless we see a combined worldwide effort furthering research into understanding these genes (and similar

other ESBLs) and the mechanisms that control them. Intensive research leading to greater understanding is imperative, for tomorrow's resistance is nothing more than the outcome of today's actions (1).

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