CHAPTER 1: INTRODUCTION.

1.1 General introduction

Extended-spectrum beta-lactamases (ESBLs) that are inhibited by clavulanic acid, belong mostly to class A of the Ambler classification scheme, conferring resistance to a wide variety of expanded-spectrum cephalosporins. To date however, a bewildering variety of enzymes have been described that can be classified as set out in Table 1-1. After being widely reported in *Enterobacteriaceae* isolates from the early 1980`s, ESBLs have been described in *Pseudomonas aeruginosa* only more recently (1, 2, 3). These enzymes described in *P. aeruginosa*, are either of the TEM- and SHV-types that are also well known in *Enterobacteriaceae*, or of the PER-type mostly originating from Turkey, or of the VEB-type from Southeast Asia and more recently, of the GES / IBC types originally reported from French Guinea, France, Greece and South Africa, respectively (4, 5, 6, 7). These five types of enzymes are remotely related, both from a genetic point of view and with respect to similarities in hydrolytic profiles. To date, CTX-M-type enzymes have not yet been described in *P. aeruginosa*.

Recent studies indicated that these enzymes may play an important role in the dissemination of antibiotic resistant bacterial isolates and may condition future choices of antibiotic regimens for treating life-threatening infections due to ESBL-producing *P. aeruginosa* (8, 9). Recent work further indicated the propensity of ESBL producing *P. aeruginosa* to establish long-term residence in the nosocomial environment, making re-infection an imminent danger (10). After the discovery of the novel ESBL, GES-2 from *P. aeruginosa*, in the Pretoria Academic Hospital (PAH) in May of 2000 (7), it was clear that class 1 integron borne ESBLs were established in the South African nosocomial setting (Discussed in detail in Chapter 3). The same *P. aeruginosa* strain subsequently caused a nosocomial outbreak in the PAH, exhibiting a 62.5% mortality

rate (11). The *P. aeruginosa* strain described during that outbreak exhibited a tendency to widely colonise and infect mostly debilitated patients, significantly increasing both their length of stay in the ICU and cost of treatment (11).

Table 1-1: Beta-lactamase classification schemes referred to in this study.

Bush group	Subgroup	Ambler molecular class	Characteristics
1		C	Mainly chromosomal located in Gram-negative bacteria but may be plasmid mediated. Confer resistance to beta-lactams (except carbapenems). Not inhibited by clavulanate.
2		A, D	Most enzymes inhibited by clavulanate (unless otherwise stated).
	2a	A	Penicillinases (narrow hydrolysis spectrum) conferring resistance to penicillins.
	2b	A	Broad-spectrum penicillinases (TEM-1, SHV-1) primarily from Gram-negative bacteria.
	2be	A	Extended-spectrum beta-lactamases conferring resistance to oxyimino-cephalosporins and monobactams.
	2br	A	Inhibitor resistant beta-lactamases (mostly TEM-types and to a lesser extent SHV derived enzymes).
	2c	A	Carbenicillinases.
	2d	D	Oxacillinases, modestly inhibited by clavulanate.
	2e	A	Cephalosporinases inhibited by clavulanate.
	2f	A	Serine active site carbapenemases, inhibited by clavulanate.
3	3a, b, c	B	Metallo-beta-lactamases conferring resistance to beta-lactams (except monobactams), not inhibited by clavulanic acid.
4			Miscellaneous unsequenced beta-lactamases that do not conform to other groups.

Data adapted from references 16 and 17.

Previous analysis of bla_{GES} genes suggested that these genetic structures did not primarily evolve from *P. aeruginosa* as the G+C content of bla_{GES-2} was 51.5%, a value which is not within the range of G+C content of *P. aeruginosa* genes (60.1 – 69.5%)

(7), this value corresponds with genes originating from the *Enterobacteriaceae*. This fact then raises the question about the origin of the GES-type genes and the possibility that they may have developed in *Enterobacteriaceae* strains including *Klebsiella pneumoniae* isolates, as was previously described in a GES-1 producing *K. pneumoniae* isolate originating from French Guinea (12). Recent developments in Japan with the finding of *bla*$_{GES-3}$ and *bla*$_{GES-4}$ (13, 14) in clinical isolates of *K. pneumoniae* strongly support this theory. The integron genetic support that these novel enzymes enjoy, not only confers resistance towards broad-spectrum beta-lactam antibiotics, but also towards unrelated classes of antibiotics such as aminoglycosides and sulphonamides as well as to several hospital disinfectants (15). Due to the composition of class 1 integrons and the simultaneous expression of all the gene cassettes comprising the integron structure (9), it is in theory possible that non-beta-lactam antibiotics may actually co-select ESBL producing *P. aeruginosa* and certain *Enterobacteriaceae*. This selection phenomenon may cause widespread nosocomial dissemination of bacterial strains harbouring these genetic structures, making treatment and control exceptionally difficult.

1.2 OBJECTIVES:

- To develop improved molecular screening and detection methods for the novel beta-lactamase GES-2 in *P. aeruginosa* isolates.
- To determine the genetic stability of *bla*$_{GES}$-type gene cassettes in class 1 integrons under antibiotic pressure in vitro.

1.3 HYPOTHESIS:

- Class 1 integron located *bla*$_{GES}$ gene cassette stability is expected to change under selective antibiotic pressure.

1.4 REFERENCES

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