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.**

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

Data adapted from references 16 and 17.

Previous analysis of *blaGES* genes suggested that these genetic structures did not primarily evolve from *P. aeruginosa* as the G+C content of *blaGES-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 *blaGES-3* and *blaGES-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 *blaGES*-type gene cassettes in class 1 integrons under antibiotic pressure in vitro.

1.3 **HYPOTHESIS:**

- Class 1 integron located *blaGES* gene cassette stability is expected to change under selective antibiotic pressure.
1.4 **REFERENCES**


7. **Poiriel, L., G. F. Weldhagen, T. Naas, C. de Champs, M. G. Dove, and P. Nordmann.** 2001. GES-2, a class A beta-lactamase from *Pseudomonas*


