The molecular basis of $\beta$-lactamase production in Gram-negative bacteria from Saudi Arabia

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Resistance to $\beta$-lactams among Gram-negative bacteria is a worldwide issue. Increased prevalence of extended-spectrum $\beta$-lactamase (ESBL)-producers and the dissemination of carbapenem-resistance genes are particularly concerning. ESBL-producing strains are common in the Kingdom of Saudi Arabia, particularly among the Enterobacteriaceae, and carbapenem resistance is on the increase, especially among the non-fermenters. $\beta$-lactamase production is a major mechanism of resistance to these agents and although $\beta$-lactamase-producing strains have been documented in the Kingdom, relatively few reports characterized the molecular basis of this production. Nevertheless, available data suggest that CTX-M (CTX-M-15 in particular) is the predominant ESBL in the Enterobacteriaceae, with SHV also being prevalent in Klebsiella pneumoniae. Carbapenem resistance in the latter is mainly due to OXA-48 and NDM-1. In Pseudomonas aeruginosa, VEB-like enzymes are the most common ESBLs, and VIM is the prevalent metallo-$\beta$-lactamase. OXA-10 extended-spectrum enzymes are also frequent. PER and GES ESBLs have been reported in Acinetobacter baumannii, and oxacillinases (OXA-23 in particular) are the dominant carbapenamases in this species.

Abbreviations: ESBL, extended-spectrum $\beta$-lactamase; MBL, metallo-$\beta$-lactamase.

Introduction

Infections due to Gram-negative bacteria are a leading cause of morbidity and mortality worldwide (Giske et al., 2008). Antimicrobial agents are a major therapeutic tool in the treatment of such infections. However, antimicrobial resistance among Gram-negative bacteria, particularly among the Enterobacteriaceae and the non-fermenters, has become one of the most serious public health concerns worldwide (Giske et al., 2008; Livermore, 2012). The global dissemination of resistance has recently received much attention, especially following reports of the international spread of multi-resistant Enterobacteriaceae, particularly strains resistant to cephalosporins due to the production of CTX-M-type extended-spectrum $\beta$-lactamases (ESBLs) and strains producing carbapenemases such as KPC and NDM (Nordmann et al., 2011; Johnson & Woodford, 2013). The spread of resistance determinants is facilitated by a number of factors, including presence on genetic mobile elements, antibiotic misuse, poor infection control practices, and increased international travel. In this context Saudi Arabia is particularly relevant as it is annually a host for more than 4 million Muslim pilgrims from over 180 countries worldwide in the Hajj and Umra seasons. In addition, up to 6 million of the Kingdom’s population are expatriates, mainly from south and east Asia, where antimicrobial resistance is rife, including to carbapenems (Yong et al., 2009). These factors could potentially make Saudi Arabia a hot spot for the collection of multidrug-resistant strains and the spread of antibiotic resistance around the world.

We previously reported on the antimicrobial resistance among the key Gram-negative bacteria in Saudi Arabia and found increased prevalence of $\beta$-lactam-resistant strains, including ESBL-producers, and carbapenem resistance (Yezli et al., 2014). However, few reports have investigated the molecular basis of $\beta$-lactamase production in the Kingdom. Here we review the available data regarding the genetic determinants for ESBL and carbapenemase production in the main Gram-negative bacteria in Saudi Arabia, namely: the Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii.

A PubMed search of relevant literature (from 1990 to 2014) was performed with a combination of the terms: ‘Pseudomonas’, ‘Acinetobacter’, ‘Enterobacteriaceae’, ‘Escherichia coli’, ‘Klebsiella’ ‘Saudi Arabia’, ‘carbapenem’, ‘$\beta$-lactam’, ‘$\beta$-lactamase’, ‘resistant’, ‘ESBL’ and ‘carbapenemase’. Reference lists of relevant articles were hand-searched to identify further material. In addition, articles were selected from relevant peer-reviewed Saudi journals where local and national data are published. We only included references where the molecular basis of $\beta$-lactamase production was characterized.
**Enterobacteriaceae**

Members of the *Enterobacteriaceae* often carry classical plasmid-encoded β-lactamases (e.g. TEM-1, TEM-2 and SHV-1) but can also express ESBLs and plasmid-mediated or chromosomal AmpC enzymes, all of which confer resistance to penicillins and extended-spectrum cephalosporins as well as monobactams (Rupp & Fey, 2003; Paterson, 2006). ESBL-producing *Enterobacteriaceae*, those of the CTX-M type in particular, are a major problem worldwide, especially due to the pandemic dissemination of CTX-M-15 in *E. coli* (Livermore, 2012). This is because ESBL-producers are often also resistant to aminoglycosides, sulfonamides and fluoroquinolones, complicating treatment choices (Carattoli, 2009; Paterson, 2006). Carbapenem resistance has now also emerged in the *Enterobacteriaceae*, mediated by carbapenemases or by combinations of ESBLs or AmpC activity together with porin loss. Plasmid-mediated carbapenemases, presently concentrated in *Klebsiella pneumoniae* and *E. coli*, include class A (KPC), B (VIM, IMP and NDM) and D (OXA-48 and -181) enzymes (Tzouvelekis et al., 2012).

*Enterobacteriaceae* are among the most commonly isolated Gram-negative bacteria from Saudi hospitals, with *E. coli* and *K. pneumoniae* being the most predominant members of this family. The reported rates of ESBL-producing strains vary between studies but are on the increase (Yezli et al., 2014). Resistance to carbapenems has emerged in the Kingdom and although still uncommon is now a major concern. Balkhy et al. (2012b) described the first outbreak of carbapenem-resistant *K. pneumoniae* in a Saudi hospital (Riyadh, central region of the Kingdom), involving 23 cases between 2009 and 2010. Further molecular characterization of 23 of the outbreak isolates showed that all isolates had an interruption in their OMP-36 gene. Five isolates had an insertion element IS903 within their OMP-36 gene and 18 had mutation/deletion of few nucleotides. All isolates carried the carbapenemase gene *blaCTX-M-15* (Balkhy et al., 2012a).

A number of studies have characterized the molecular basis of β-lactamase production in *E. coli* and *K. pneumoniae* from Saudi Arabia (Table 1). An investigation into 160 ESBL-producing *E. coli* and *K. pneumoniae* isolates collected in 2009 from Riyadh found CTX-M (mainly CTX-M-15) in 93.1 % of the isolates. SHVs (SHV-1 and SHV-5) were reported in 6.9 % of the isolates (Shibl et al., 2012). Somily et al. (2012) on the other hand found that TEM was the most common ESBL among *E. coli* and *K. pneumoniae*, with increasing prevalence of CTX-M type among *E. coli* in particular. Of the 11 231 *E. coli* and *K. pneumoniae* isolated from Riyadh during a 5 year period (2006–2010), 1160 (10.3 %) were ESBL-producers (mainly *E. coli*). TEM was found in 60 % of the ESBL-producers. A noticeable increase in the prevalence of CTX-M during the study period was observed for *E. coli* (from 5.1 to 25.3 %) but not *K. pneumoniae* (from 6.4 to 7.4 %). Other class types, namely class C, non-A and non-C, were found in fewer than 10 % of the isolates (Somily et al., 2012).

In another report, Marie et al. (2013) collected 4250 *E. coli* and *K. pneumoniae* isolates from a hospital in Riyadh between 2010 and 2011; they found high carbapenem resistance (30–50 %) and 72 % produced β-lactamases. ESBLs were detected more frequently in the 3358 *E. coli* isolates than carbapenemases (68 vs 53 %), while for the 892 *K. pneumoniae* isolates the frequency of detection of ESBLs and carbapenemases was similar (63 %). CTX-M was the most frequent ESBL (63.5 %), with CTX-M-9 and CTX-M-1 being most prevalent. TEM and SHV were detected in 58 and 27.3 % of the isolates, respectively, while the combination of the three ESBLs (CTX-M, TEM and SHV) was found in 29 % of the isolates.

Two studies from the eastern region of the Kingdom reported the predominance of the CTX-M genotype among ESBL-producing *E. coli* isolates (Hassan et al., 2013; Bindayna et al., 2010). Hassan et al. (2013) found that the proportions of their 139 ESBL-producing *E. coli* collected from a university hospital between 2007 and 2009 that carried CTX-M, TEM and SHV genes were 76, 50 and 22 %, respectively. CTX-M, TEM and SHV were also found in various combinations in the isolates, and all were found in 10 % of the isolates. Similarly, Bindayna et al. (2010) found CTX-M, TEM and SHV in, respectively, 76, 12 and 1 % of their 84 ESBL-producing *E. coli* isolated in 2006. The combination of CTX-M and TEM was detected in some isolates, while no other combinations were noted. Recently, CTX-M-15 was reported in all *E. coli* with reduced susceptibility to ertapenem isolated from Riyadh between 2011 and 2013 (Zowawi et al., 2014).

The predominance of the CTX-M genotype among ESBL-producing *K. pneumoniae* isolated from the Kingdom has also been reported in some studies but the majority show the prevalence of SHV (Table 1). One study in 2007–2009 found CTX-M and SHV in, respectively, 91 and 86 % of ESBL-producing *K. pneumoniae*, while TEM was found in 48 % of the isolates (Hassan et al., 2013). Another study, in 2006 (Bindayna et al., 2010) found CTX-M and SHV equally prevalent (in 43.7 % of the isolates) and TEM in 37.5 % of the isolates. All three genotypes were present in 12–36 % of the isolates (Hassan et al., 2013; Bindayna et al., 2010). Surprisingly, Al-Agamy et al. (2009a) found all three genotypes in all their 16 ESBL-producing *K. pneumoniae* isolated from neonatal patients during an outbreak in 2009 in a hospital in the eastern region of the Kingdom. Because all isolates carried the CTX-M-15, SHV-1 and TEM-1 genes and had the same antimicrobial resistance pattern, it was likely that they all represented a single clone. Nevertheless, this was the first report of the *blaCTX-M-15* gene in *K. pneumoniae* in Saudi Arabia.

In another study, Al-Agamy et al. (2009b) identified a high ESBL rate of 55 % among 400 *K. pneumoniae* isolated from two hospitals in Riyadh in 2007. SHV was detected in most (97.3 %) of the ESBL-producers, followed by TEM (84.1 %) and CTX-M (34.1 %). Both the CTX-M-15-like gene (CTX-M-1 group) and the CTX-M-14/18-like gene (CTX-M-9


group) were detected. Predominance of SHV and TEM among ESBL-producing K. pneumoniae was in agreement with another report from the Al-Qassim region, located near Riyadh (Tawfik et al., 2011). In this report, ESBL production was found in 25.6% of the 430 K. pneumoniae isolated in 2008, all of which were sensitive to imipenem and ticarcillin. The prevalence of SHV, TEM and CTX-M among the ESBL-producers was 91, 71 and 36.4%, respectively. In the SHV group, the variants SHV-12 and SHV-5 were the most prevalent, as is the case worldwide (Paterson et al., 2003; Villegas et al., 2008; Ryoo et al., 2005). Other non-ESBL SHVs were also detected at lower rates, including SHV-1, SHV-11 and SHV-85, found in six, four and two isolates, respectively. SHV-12 has already been reported in Saudi Arabia from a K. pneumoniae that caused an outbreak in a neonatal unit in 2008 (Al-Obeid et al., 2008), while the Tawfik et al. (2011) report was the first description of SHV-5, SHV-11 and SHV-85 in the Kingdom. The study was also the first description of CTX-M-14 in Saudi Arabia, which was found in 5% of the 40 CTX-M-producing isolates, while the remaining 95% belonged to the more common CTX-M-15.

Recently, Al-Qahtani et al. (2014) also reported SHV to be predominant among ESBL-producing K. pneumoniae isolated between 2011 and 2012 from a hospital in Riyadh, accounting for 92% of the 37 isolates. However, they reported CTX-M also to be highly prevalent in 86.5% of the isolates, in contrast with TEM, found in only 54% of the isolates. Most SHVs were SHV-1 and SHV-12, found in, respectively, 21 and 11 of the 34 SHV isolates. SHV-5 represented 5.8% of the SHV isolates. DNA sequencing of the TEM genes indicated that blaTEM genes were all blaTEM-1. CTX-M-15 was found to be the most prevalent ESBL gene, detected in 75% of the isolates. The study also documented the first report of CTX-M-3, CTX-M-27, CTX-M-57 and CTX-M-82 in K. pneumoniae in the Kingdom. Recently, CTX-M-15 was reported in 70% of K. pneumoniae with reduced susceptibility to ertapenem isolated from Riyadh between 2011 and 2013 (Zowawi et al., 2014).

Carbapenemase production

Limited data are available on the molecular basis of carbapenem resistance in Enterobacteriaceae from the Kingdom. All these studies were from Riyadh and report the emergence of OXA-48 and NDM-1, especially among K. pneumoniae. Al-Agamy et al. (2013) characterized nine resistant K. pneumoniae isolated from Riyadh hospitals in 2011. They detected OXA-48 and NDM carbapenemases in 77.7 and 22.2% of the isolates, respectively. None of the isolates harboured KPC, VIM or IMP, but all carried the SHV gene. blaCTX-M group1 genes were detected in eight of the nine isolates. In another study, of the 60 K. pneumoniae with reduced susceptibility to carbapenem collected in 2011 (Shibl et al., 2013), 47 (78%) carried the OXA-48 gene, while NDM-1 and VIM were found in 20 and 1.6% of the isolates, respectively. None of the isolates harboured a combination of the three resistance genes or KPC. Thirty-seven isolates (61%) were positive for blaCTX-M, and all belonged to CTX-M group1. Twenty-nine (48%) were positive for both blaCTX-M and blaOXA-48, and eight (13.3%) were positive for blaCTX-M and blaNDM-1. The blatem and blaSHV genes were detected in 28 and 65% of the isolates, respectively.

Marie et al. (2013) found NDM in 55% of the 3060 E. coli and K. pneumoniae isolates from a hospital in Riyadh between 2010 and 2011. IMP and VIM were identified in, respectively, 9 and 7% of the isolates, while KPC and OXA-48 were not detected in any of the isolates. The combination of NDM, VIM and IMP was found in 4% of the isolates, and in general 22% of the isolates contained both ESBLs and metallo-β-lactamases (MBLs). Al-Qadheeb et al. (2010) reported the first case of KPC-producing K. pneumoniae in the Kingdom, isolated from a 75-year-old immune-compromised patient in Riyadh. The strain was multidrug-resistant, including to carbapenems and colistin, and evolved in the same patient from susceptible to resistant to tigecycline during the course of treatment. A recent study investigating isolates with reduced susceptibility to ertapenem collected from a hospital in Riyadh between 2011 and 2013 found NDM in one of the two E. coli isolates (Zowawi et al., 2014). The same study reported OXA-48 in 77.5% of the 40 K. pneumoniae isolates and NDM in 25%. Six isolates co-harboured blaOXA-48 and blaNDM.

In summary, data suggest that in the Kingdom of Saudi Arabia CTX-M (CTX-M-15 in particular) is the predominant ESBL in E. coli, correlating with international scenarios (Livermore, 2012), while other ESBLs such as TEM and SHV have been reported. For K. pneumoniae, the main ESBLs are CTX-M (CTX-M-15 in particular) and SHV. TEM has also been reported in this species. Carbapenem resistance in K. pneumoniae is mainly due to OXA-48 and NDM-1, although VIM and KPC have also been reported. The emergence of OXA-48 and NDM carbapenemases in Saudi Arabia may reflect the extensive population flow between the Kingdom, other parts of the Middle East, where OXA-48 is widespread, and the Indian subcontinent, where NDM is common (Nordmann et al., 2011). On the other hand, the low rate of VIM and KPC suggests that so far these are not major sources of carbapenemases in Riyadh at least.

P. aeruginosa

P. aeruginosa is intrinsically resistant to many β-lactams but is susceptible to carboxyl- and piperazine-penicillins, to several oxyimino cephalosporins and monobactams, and to carbapenems except ertapenem (Strateva & Yordanov, 2009). Most of this resistance is mediated via important mutational mechanisms, including derepression of AmpC β-lactamase, upregulation of active efflux, and loss of the ‘carbapenem-specific’ outer membrane porin, OprD. In addition, many diverse acquired β-lactamases have been reported in the species, including several class A, B and D β-lactamases, such as VEB-, PER-, GES-, TEM-, SHV- and
<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Organism (n isolates)</th>
<th>Phenotype*</th>
<th>Most common $\beta$-lactamases†</th>
<th>Other $\beta$-lactamases‡</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern region</td>
<td>2006</td>
<td>E. coli (84)</td>
<td>ESBL + ve</td>
<td>CTX-M (76%)</td>
<td>TEM (12%), SHV (1%)</td>
<td>Bindayna et al. (2010)</td>
</tr>
<tr>
<td>Eastern region</td>
<td>2007–2009</td>
<td>E. coli (139)</td>
<td>ESBL + ve</td>
<td>CTX-M (76%)</td>
<td>TEM (50%), SHV (22%)</td>
<td>Hassan et al. (2013)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2009</td>
<td>E. coli + K. pneumonae (160)</td>
<td>ESBL + ve</td>
<td>CTX-M (93.1%) [of the 149 CTX-M; CTX-M-15 (96.6%), CTX-M-14 (3.3%)]</td>
<td>SHV (6.9%) [of the 11 SHV; SHV-12 (63.6%), SHV-5 (36.3%)]</td>
<td>Shibl et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2011–2013</td>
<td>E. coli (2)</td>
<td>Ert-Rd</td>
<td>CTX-M-15 (100%), NDM (50%)</td>
<td>–</td>
<td>Zowawi et al. (2014)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2006–2010</td>
<td>E. coli + K. pneumonae (1160)</td>
<td>ESBL + ve</td>
<td>TEM (60%)</td>
<td>CTX-M (5.1–25.3%), class C, non-A and non-C (&lt;10%)</td>
<td>Somily et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010–2011</td>
<td>E. coli + K. pneumonae (3060)</td>
<td>β-lactamase + ve</td>
<td>CTX-M (63.5%) [of the 2698 CTX-M; CTX-M-9 (57%), CTX-M-1 (32%), CTX-M-2 (11%), NDM (55%)]</td>
<td>TEM (58%), SHV (27.3%), IMP (9%), VIM (7%)</td>
<td>Marie et al. (2013)</td>
</tr>
<tr>
<td>Eastern region</td>
<td>2006</td>
<td>K. pneumonae (16)</td>
<td>ESBL + ve</td>
<td>CTX-M (43.7%), SHV (43.7%)</td>
<td>TEM (37.5%)</td>
<td>Bindayna et al. (2010)</td>
</tr>
<tr>
<td>Eastern region</td>
<td>2007</td>
<td>K. pneumonae (16)</td>
<td>ESBL + ve</td>
<td>CTX-M (100% CTX-M-15), TEM (100% TEM-1), SHV (100% SHV-1)</td>
<td>–</td>
<td>Al-Agamy et al. (2009a)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2007</td>
<td>K. pneumonae (220)</td>
<td>ESBL + ve</td>
<td>SHV (97.3%)</td>
<td>TEM (84.1%), CTX-M (34.1%) [of the 75 CTX-M; CTX-M-1-like (60%), CTX-M-9-like (40%)]</td>
<td>Al-Agamy et al. (2009b)</td>
</tr>
<tr>
<td>Eastern region</td>
<td>2007–2009</td>
<td>K. pneumonae (90)</td>
<td>ESBL + ve</td>
<td>CTX-M (91%)</td>
<td>SHV (86%), TEM (48%)</td>
<td>Hassan et al. (2013)</td>
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<tr>
<td>Central region</td>
<td>2008</td>
<td>K. pneumonae (110)</td>
<td>ESBL + ve</td>
<td>SHV (91%) [of the 100 SHV; SHV-12 (68%), SHV-5 (20%), SHV-1 (6%), SHV-11 (4%), SHV-85 (2%)]</td>
<td>TEM (71%), CTX-M (36.4%) [of the 40 CTX-M; CTX-M-15 (95%), CTX-M-14 (5%)]</td>
<td>Tawfik et al. (2011)</td>
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<tr>
<td>Riyadh</td>
<td>ND</td>
<td>K. pneumonae</td>
<td>Car-R</td>
<td>KPC (100%)</td>
<td>–</td>
<td>Al-Qadheeb et al. (2010)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2009–2010</td>
<td>K. pneumonae (23)</td>
<td>Car-R</td>
<td>OXA-48 (100%)</td>
<td>–</td>
<td>Balkhy et al. (2012a)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2011</td>
<td>K. pneumonae (60)</td>
<td>Car-Rd</td>
<td>OXA-48 (78%)</td>
<td>SHV (65%), CTX-M (61%), TEM (28.3%), NDM-1 (20%), VIM (1.6%)</td>
<td>Shibl et al. (2013)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2011</td>
<td>K. pneumonae (9)</td>
<td>Car-R</td>
<td>SHV (100%), OXA-48 (77.7%)</td>
<td>CTX-M (88.8%), NDM-1 (22.2%)</td>
<td>Al-Agamy et al. (2013)</td>
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<tr>
<td>Riyadh</td>
<td>2011–2012</td>
<td>K. pneumonae (37)</td>
<td>ESBL + ve</td>
<td>SHV (92%) [of the 34 SHV; SHV-1 (61.7%), SHV-12 (32.3%), SHV-5 (5.8%)]</td>
<td>CTX-M (86.5%) [of the 32 CTX-M; CTX-M-15 (87.5%), CTX-M-3 (3.1%), CTX-M-57 (3.1%), CTX-M-82 (3.1%), CTX-M-27 (3.1%), TEM (54%) [of the 20 TEM, TEM-1 (100%)]</td>
<td>Al-Qahtani et al. (2014)</td>
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<tr>
<td>Location</td>
<td>Date</td>
<td>Organism (n isolates)</td>
<td>Phenotype*</td>
<td>Most common β-lactamases†</td>
<td>Other β-lactamases†</td>
<td>Reference</td>
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<tr>
<td>Riyadh</td>
<td>2005</td>
<td>K. pneumoniae</td>
<td>ESBL + ve</td>
<td>SHV-12</td>
<td>–</td>
<td>Al-Obeid et al. (2008)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2011–2013</td>
<td>K. pneumoniae (40)</td>
<td>Ert-Rd</td>
<td>OXA-48 (77.5 %), CTX-M-15 (70 %)</td>
<td>NDM (25 %)</td>
<td>Zowawi et al. (2014)</td>
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<tr>
<td>National</td>
<td>2006–2007</td>
<td>A. baumannii (20)</td>
<td>Ert-R</td>
<td>OXA-89 (25 %)</td>
<td>OXA-131‡ (20 %), OXA-66 (15 %), OXA-90‡ (10 %), OXA-91 (10 %), OXA-132‡ (10 %), OXA-75 (5 %), OXA-130‡ (5 %)</td>
<td>Alsultan et al. (2009)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2006–2008</td>
<td>A. baumannii (253)</td>
<td>MDR</td>
<td>OXA-51-like (100 %) [of the 253 OXA-51-like; OXA-66 (62.3 %), OXA-69 (19.1 %), OXA-132 (7.6 %), OXA-79, -82, -94, -95, -98, -131 (11 %)], OXA-23 (100 %)</td>
<td>OXA-58 (2 %)</td>
<td>Aly et al. (2014)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010</td>
<td>A. baumannii (27)</td>
<td>Mer-R</td>
<td>OXA-51 (100 %), PER (48.1 %)</td>
<td>OXA-23 (60 %), OXA-40 (3.7 %), GES-1 (22 %), GES-11 (11.1 %), GES-5 (3.7 %)</td>
<td>Ribeiro et al. (2012)</td>
</tr>
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<td>National</td>
<td>2008–2011</td>
<td>A. baumannii (196)</td>
<td>Car-R</td>
<td>VIM (93 %)</td>
<td>OXA-23 (55 %), OXA-40 (30 %)</td>
<td>Alsultan et al. (2013)</td>
</tr>
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<td>2008–2011</td>
<td>A. baumannii (75)</td>
<td>Random (65 % Car-R)</td>
<td>VIM (96 %)</td>
<td>OXA-23 (56 %), OXA-40 (12 %)</td>
<td>Alsultan et al. (2013)</td>
</tr>
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<td>Eastern region</td>
<td>2010–2012</td>
<td>A. baumannii (46)</td>
<td>CR-R</td>
<td>OXA-51 (100 %)</td>
<td>OXA-23 (80 %)</td>
<td>Abdalhamid et al. (2014)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2011</td>
<td>A. baumannii (28)</td>
<td>CR-R</td>
<td>OXA-51-like (100 %)</td>
<td>OXA-23 (50 %)</td>
<td>Alsultan (2012)</td>
</tr>
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<td>ND</td>
<td>ND</td>
<td>P. aeruginosa (1)</td>
<td>CR-R</td>
<td>VIM-2</td>
<td>–</td>
<td>Guerin et al. (2005)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2007</td>
<td>P. aeruginosa (135)†</td>
<td>MBL + ve</td>
<td>VIM (100 %)</td>
<td>–</td>
<td>Al-Agamy et al. (2009c)</td>
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<td>Western region</td>
<td>2009–2010</td>
<td>P. aeruginosa (31)</td>
<td>MBL + ve</td>
<td>IMP (22.6 %)</td>
<td>VIM (19.4 %)</td>
<td>Asghar (2012)</td>
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<tr>
<td>Riyadh</td>
<td>2010</td>
<td>P. aeruginosa (35)</td>
<td>ESC-Ns</td>
<td>VEB (48.5 %), VIM (42.8 %)</td>
<td>OXA-10 (40 %), GES (14.2 %)</td>
<td>Tawfik et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010</td>
<td>P. aeruginosa (25)</td>
<td>ESBL + ve</td>
<td>VEB (68 %), VIM</td>
<td>OXA-10 (56 %), GES (20 %)</td>
<td>Tawfik et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010</td>
<td>P. aeruginosa (15)</td>
<td>MBL + ve</td>
<td>VIM (100 %)</td>
<td>OXA-10 (33.3 %)</td>
<td>Tawfik et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010</td>
<td>P. aeruginosa (39)</td>
<td>CAZ-R</td>
<td>VEB (51.2 %), VIM (41 %)</td>
<td>OXA-10 (41 %), GES (12.8 %)</td>
<td>Al-Agamy et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010</td>
<td>P. aeruginosa (23)</td>
<td>ESBL + ve</td>
<td>VEB (87 %)</td>
<td>OXA-10 (43.5 %), GES (21.7 %)</td>
<td>Al-Agamy et al. (2012)</td>
</tr>
<tr>
<td>Riyadh</td>
<td>2010</td>
<td>P. aeruginosa (16)</td>
<td>MBL + ve</td>
<td>VIM (100 %)</td>
<td>OXA-10 (37.5 %)</td>
<td>Al-Agamy et al. (2012)</td>
</tr>
</tbody>
</table>

ND, Not determined.

*Ert, Ertapenem; Car, carbapenem; Caz, ceftazidime; R, resistant; Rd, reduced susceptibility; ESC-Ns, extended-spectrum cephalosporins, non-susceptible; MDR, multidrug-resistant (resistant to at least three classes of antibiotics).

†Carbapenemases are shown in bold type.

‡Novel OXA-15-like; probably a single clone.
OXA-types (Strateva & Yordanov, 2009; Lister et al., 2009). Carbapenem resistance in *P. aeruginosa* is mostly due to MBLs, including IMP, VIM, SPM, GIM, AIM and DIM enzymes, although other enzymes, including KPC, GES and OXA variants, have been recorded (Sacha et al., 2008; Wang et al., 2010; Juan Nicolau & Oliver, 2010). Multiple mechanisms of resistance often exist in combination (Strateva & Yordanov, 2009; Lister et al., 2009).

*P. aeruginosa* is one of the most commonly isolated pathogens in Saudi hospitals and reports from the Kingdom indicate a steady increase in resistance to relevant antipseudomonal drugs and worrying rates of multidrug resistance, including strains with ESBLs and carbapenemases (Yezli et al., 2014). ESBLs have been found in 3–40% of clinical *P. aeruginosa* (Kader et al., 2004; Kader & Angamuthu, 2005) and in, respectively, 59 and 69% of those that are ceftazidime-resistant and extended-spectrum cephalosporin-non-susceptible (Tawfik et al., 2012; Al-Agamy et al., 2012). Carbapenem-hydrolysing enzymes have also been documented, including MBLs (Guerin et al., 2005; Al-Agamy et al., 2012; Tawfik et al., 2012). A recent national study found 16% of isolates were resistant to imipenem, although resistance rates of up to 34% for meropenem and up to 56% for imipenem have been documented among isolates collected from individual healthcare facilities between 2009 and 2010 (Saeed et al., 2010; Abo-Shadi et al., 2012).

The molecular basis of ESBL and carbapenemase production in *P. aeruginosa* isolated from the Kingdom was reported in a limited number of studies (Table 1). In one study (Guerin et al., 2005), an MBL-producing strain of *P. aeruginosa* was recovered from a human immunodeficiency virus (HIV)-positive Saudi male who developed a urinary tract infection in France. It was believed that the patient was colonized by the strain before arriving in France. The strain, RZ01, was resistant to most antipseudomonal β-lactams as well as aminoglycosides and fluoroquinolones, but not to colistin and fosfomycin. Molecular characterization of the strain revealed the presence of VIM-2. In another report, Al-Agamy et al. (2009c) detected *bla*<sub>VIM</sub>-like determinants as part of a gene cassette of class 1 integrons in all their MBL-producing *P. aeruginosa* isolates from a hospital in Riyadh in 2007. These MBL-producers represented 16.3% of the 135 isolates collected; all were resistant to imipenem (MIC ≥ 32 μg ml<sup>−1</sup>) and polymyxin B, and 77% were resistant to aztreonam. None of the isolates carried genes for IMP, GIM or SIM. Because of the similarities in results for the MBL-producers, it is likely that they represented a single clone.

One study reported IMP to be more prevalent than VIM among MBL-producing *P. aeruginosa* isolated between 2009 and 2010 from hospitals in Mecca, located in the western region of the Kingdom (Asghar, 2012). Of the 31 MBL-producers, IMP was detected in 22.6% while VIM was detected in 19.4%. However, other reports from Riyadh suggest that VIM is the dominant carbapenemase in *P. aeruginosa* from that region. For example, Tawfik et al. (2012) investigated 35 extended-spectrum cephalosporin-non-susceptible *P. aeruginosa* collected during 2010. They identified VIM in 42.8% of the isolates and in 100% of the MBL-producers. IMP, GIM, SIM, SPM and NDM were not detected. The study was also the first report of the ESBLs VEB and GES, and OXA-10 extended-spectrum enzyme in Saudi Arabia. VEB-1, OXA-10 and GES were found in 48.5, 40 and 14.2% of the isolates respectively. PER, TEM, SHV and CTX-M were not detected in any of the isolates. The OXA-10-like gene was concomitant with VEB, GES and/or VIM. Eight (22.8%) isolates harboured OXA-10 with VEB (imipenem MIC 6–8 mg l<sup>−1</sup>), while five (14.2%) isolates harboured OXA-10 with VIM (imipenem MIC ≥ 32 mg l<sup>−1</sup>), and one (2.8%) isolate contained OXA-10, VEB and GES (imipenem MIC 8 mg l<sup>−1</sup>).

A similar pattern of results was reported in another study, investigating 39 ceftazidime-resistant *P. aeruginosa* isolates from another Riyadh hospital in 2010 (Al-Agamy et al., 2012). VEB-1, OXA-10 and GES were found in 51.2, 41 and 12.8% of the isolates, respectively, while VIM was detected in 41% of the isolates and in 100% of the MBL-producers. None of the following enzymes was detected: PER, TEM, SHV, CTX-M, IMP, GIM, SIM, SPM and NDM. The OXA-10-like gene was found in 16 isolates in combinations with VEB, GES and/or VIM. Nine (23%) isolates harboured OXA-10 with VEB (imipenem MIC 6–8 mg l<sup>−1</sup>), while six (15.3%) isolates carried OXA-10 with VIM genes (imipenem MIC ≥ 32 mg l<sup>−1</sup>), and one (2.5%) isolate contained OXA-10, VEB and GES (imipenem MIC 8 mg l<sup>−1</sup>).

In summary, data from the Kingdom suggest that VEB-like enzymes are the most common ESBLs in *P. aeruginosa* from Saudi Arabia, in accordance with reports from the Middle East, South East Asia, and parts of Europe (Strateva & Yordanov, 2009; Woodford et al., 2008). OXA-10-like enzymes are also frequent, as is the case in several countries (Mirsalehian et al., 2010; Strateva & Yordanov, 2009). VIM appears to be the most common MBL, as is also the case in many countries worldwide (Lagatolla et al., 2004; Valenza et al., 2010; Lee et al., 2003; Strateva & Yordanov, 2009).

### A. baumannii

*A. baumannii* is resistant to many β-lactams. Resistance to cephalosporins is often associated with the upregulation of a chromosomal *ampC* gene by insertion sequences, predominantly ISA<sub>b</sub>al (Hamidian & Hall, 2013), whereas carbapenem resistance may be due to upregulation of the chromosomally mediated *bla*<sub>OXA-51-like</sub> β-lactamase gene against, generally, ISA<sub>b</sub>al, or acquisition of further OXA-carbapenemases or of class B MBLs, including IMP, VIM, SIM and NDM types (Gordon & Wareham, 2010; Perez et al., 2007). Other mechanisms of β-lactam resistance identified in occasional *A. baumannii* isolates include class A ESBLs, including TEM, CTX-M and GES, changes in penicillin-binding proteins, alterations in porin proteins,
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and activation of efflux pumps (Gordon & Wareham, 2010; Perez et al., 2007).

A. baumannii is a successful nosocomial pathogen and is highly prevalent in Saudi intensive care units (ICUs), causing numerous outbreaks, many of them involving multidrug-resistant strains (Mah et al., 2001; Manzar, 2004; Al Shirawi et al., 2006; Bukhary et al., 2005; Yezli et al., 2014). There is evidence that resistance is increasing to many antibiotics in the Kingdom, including to carbapenems, which nevertheless remain the treatment of choice for most A. baumannii infections (Yezli et al., 2014). Although a recent nationwide survey of 2228 A. baumannii isolates collected in 2009 found 5.4% were resistant to imipenem (Memish et al., 2012), rates at individual units can be much higher. For instance, two reports found that around 90% of A. baumannii isolated from ICU patients in Riyadh between 2004 and 2009 were resistant to carbapenems (Saeed et al., 2010; Al Johani et al., 2010). ESBL production is also common. Kader et al. (2004) asserted that 37.5% of the Acinetobacter from urine cultures of patients at their hospital in 1999–2002 were ESBL-producing. While β-lactam resistance appears to be frequent, the underlying mechanisms are little studied in the Kingdom (Table 1).

In a recent multicentre study including 37 Saudi hospitals, Alsultan et al. (2013) investigated the molecular basis of resistance in 196 carbapenem-resistant A. baumannii isolated between 2008 and 2011. A high proportion of isolates (99%) carried genes for at least one acquired carbapenem-hydrolysing β-lactamase. VIM was found in 93% of the isolates, while OXA-23 and OXA-40 genes were present in 55 and 30% of the isolates, respectively. VIM was also predominant among 75 randomly selected A. baumannii isolates (65% of which were carbapenem resistant), accounting for 96% of the isolates. This confirms results of previous studies that showed that the number of isolates carrying a gene for the VIM-type enzyme is much higher than the number of isolates phenotypically resistant to carbapenems (Franklin et al., 2006; Peleg et al., 2005).

The results of Alsultan et al. (2013) are unusual by international standards, where the most frequent carbapenem-hydrolysing β-lactamases in A. baumannii are oxacillinases (OXA-23, OXA-40, OXA-58 and OXA-143) (Nordmann, 2010). Many reports from the Kingdom confirmed the prevalence of oxacillinases (particularly OXA-23) among carbapenem-resistant A. baumannii. For example, one study detected OXA-23 in half of the 28 carbapenem-resistant A. baumannii isolates collected in 2011, while the intrinsic OXA-51-like gene was detected in all isolates (Alsultan, 2012). Ribeiro et al. (2012) investigated 27 meropenem-resistant A. baumannii isolates collected from a hospital in Riyadh in 2010 and reported predominance of OXA-23 occurring across different sequence types. The 27 strains were distributed in eight sequence types, and only 11 isolates belonged to the two most prevalent clonal complexes in the world, CC1 (ST1, ST7) and CC2 (ST2). Double disc synergy tests using ceftazidime and clavulanic acid indicated the production of a class A ESBL in 13 isolates, contrasting with the detection of genes encoding enzymes PER-1 (n=13), GES-1 (n=6), GES-5 (n=1) and GES-11 (n=3) in 23 strains. The intrinsic OXA-51 was present in all isolates, and OXA-23 and OXA-40 were produced by 16 and 1 isolate, respectively. The ISAba1 element was systematically found upstream of all the carbapenemases.

Abdalhamid et al. (2014) found a higher rate of OXA-23 in their carbapenem-resistant A. baumannii isolated from the eastern region of the Kingdom. Of the 141 isolates collected between August 2010 and September 2012, 32.6% were resistant to carbapenems. OXA-23 was detected in 80% of the carbapenem-resistant isolates, while the OXA-51 gene used as a marker for A. baumannii was found in all isolates. blaOXA-23 was encoded downstream of the ISAba1 and this was the main mechanism of resistance in these isolates. No blaPER, blavim or blandm1 was detected in any of the isolates. Alsultan et al. (2009) investigated 20 ertapenem-resistant A. baumannii isolates from diabetic patients collected between 2006 and 2007 from hospitals and medical centres in Saudi Arabia. Nine isolates (45%) carried four novel OXA-51-like β-lactamases, designated OXA-90, OXA-130, OXA-131 and OXA-132. In addition, OXA-89, OXA-66, OXA-91 and OXA-75 genes were found in 25, 15, 10 and 5% of the isolates, respectively.

Recently, Aly et al. (2014) investigated the genetic diversity of the OXA-51-like genes among 253 multidrug-resistant (resistant to at least three classes of antibiotics) A. baumannii collected between 2006 and 2008 in Riyadh. They reported that all isolates contained OXA-51-like and OXA-23 genes, 2% had the OXA-58 gene, and none had the OXA-40 gene. Further molecular testing revealed that the OXA-51-like genes clustered into four main groups: OXA-66 (62.3%), OXA-69 (19.1%), OXA-132 (7.6%) and other OXA-51-like genes (found in 11% of the isolates).

In summary, data from the Kingdom suggest that PER and GES are common ESBLs in A. baumannii, as is the case in many other countries (Gordon & Wareham, 2010). OXA β-lactamases (OXA-23 in particular) are the dominant carbapenemases in this species, as reported elsewhere in the world (Walther-Rasmussen & Høiby, 2006; Gordon & Wareham, 2010). For the intrinsic OXA-51, OXA-66 appears to be the major OXA-51-like gene in the Kingdom, consistent with reports from the USA, South America, Turkey and China (Aly et al., 2014). VIM has also been documented in A. baumannii from Saudi Arabia, similar to many reports from the Asia Pacific region and Latin America (Fritsche et al., 2005).

Summary

β-Lactam resistance among Gram-negative bacteria is a serious worldwide concern, with increasing rates of ESBL-producers and the emergence and dissemination of carbapenem-resistance genes. Because of its geographical
Generally, the mechanisms of β-lactamase production in E. coli are documented in this species. Although the Kingdom has high rates of ESBL-producers and is experiencing a progressive increase in prevalence of carbapenem-resistant strains, limited data are available on the molecular basis of β-lactamase production in the country.

Available information indicates that for E. coli the predominant ESBL is CTX-M (CTX-M-15 in particular), while other ESBLs such as TEM and SHV have also been reported in the species. For K. pneumoniae, the main β-lactamases are SHV (SHV-12, SHV-5 and SHV-1 in particular), CTX-M (CTX-M-15 in particular) and TEM. The emergence of OXA-48 and NDM-1 conferring carbapenem resistance in K. pneumoniae has been documented, while prevalence of VIM and KPC is rare so far. In P. aeruginosa, VEB- and OXA-10-like enzymes are the most common β-lactamases, with GES being reported. VIM is the most prevalent MBL in P. aeruginosa. In A. baumannii, PER and GES ESBLs have been reported, while OXA-23 appears to be the dominant carbapenemase. VIM MBL has also been documented in this species.

Generally, the mechanisms of β-lactamase production in P. aeruginosa and A. baumannii as well as ESBL production in E. coli and K. pneumoniae are in accordance with international scenarios. This may be due in part to the introduction and dissemination of resistance determinants imported from across the globe as a consequence of international travel, especially during the mass gathering events common in the Kingdom. The emergence of OXA-48 and NDM carbapenemases in Saudi Arabia may reflect the extensive population flow between the Kingdom, other parts of the Middle East, where OXA-48 is widespread, and the Indian subcontinent, where NDM is common. This is in contrast with the currently low prevalence of other resistance mechanisms such as KPC common in countries geographically further away from and with relatively fewer visitors to the Kingdom, such as the United States. It is worth noting that few studies have investigated carbapenemase production in the Enterobacteriaceae in Saudi Arabia and most of these were from a single region of the country. It is possible that further national investigations may reveal resistance mechanisms such as KPC to be more prevalent in the Kingdom than is currently reported.

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