*INT. J. BIOL. BIOTECH.*, 18 (4) : 665-672, 2021.

# DISSEMINATION OF CONJUGATIVE INCF PLASMIDS AND INSERTION SEQUENCES ELEMENTS ASSOCIATED WITH ANTIMICROBIAL RESISTANCE IN SAUDI ARABIA: A SERIOUS HEALTH THREAT

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## ABSTRACT

This report aimed to document the genomic characterization and the genetic profile analysis related to the dissemination of beta-lactams and vancomycin through Mobile Genetic Elements (plasmids, insertion sequences and transposons) in different settings including clinical and sewage water. PubMed and Medline database were used to search the relevant literature from 2004 to 2020 and data of interest from Central for Diseases Control and Prevention and those predicted by the WHO were also included. Genetic profile analysis showed diversity of Mobile Genetic Elements (MGEs) with the predominance of conjugative IncF plasmids types and insertion sequences elements carrying cephalosporins, carbapenem and vancomycin resistance genes. The propagation of these genetic elements constitutes a veritable global health threat and urges to establish new strategic plan for combating antibiotic resistance in Saudi Arabia.

**Key words**: Antibiotic resistance genes, mobile genetic elements, Saudi Arabia.

## INTRODUCTION

Antimicrobial resistance (AMR) in bacteria is emerging and spreading rapidly worldwide. This phenomenon is nowadays affecting public and animal health dramatically on a global level. Unfortunately, on the one hand the current dependence on antibiotics- whether to treat, prevent, or stimulate food animal growths and on the other hand the self-medication with antibiotics without requiring medical consultation have exponentially increased this resistance in many countries from Middle East including Saudi Arabia (Alawi and Darvesh, 2016; Zowawi, 2016; Zakai, 2019**).**

Multi-Drug-Resistant (MDR) bacteria have been an epidemiological concern as they may spread locally, regionally or globally through individual contacts, poor sanitation, travel or food chain. Thus, leading to serious environmental and public health problems in the community (Aslam *et al*., 2018; Leangapichart *et al*., 2016). Molecular analyses have revealed that widespread of these bacteria has commonly been achieved by the acquisition of antibiotic resistance genes (ARGs). The dissemination of resistance genes is largely due to the actions of mobile genetic elements (MGE), and the antibiotic resistance is often spread via mobile genetic elements, which tend to code for genes providing resistance against multiple antibiotics (Paritridge *et al*., 2018; Davies and Davies, 2010). MGEs are sequences of genetic material that promote intracellular DNA mobility (e.g., from the chromosome to a plasmid or between plasmids) as well as those that enable intercellular DNA mobility. MGEs can change places on a chromosome, and be exchanged between chromosomes, between bacteria, and even between species. Insertion sequences (IS) and transposons (Tn) are examples of MGEs that have the capacity to move themselves and associated resistance genes randomly in the same or different DNA molecules within a single cell (Foxmen, 2012; Paritridge *et al*., 2018). Plasmid transmission is another important way of intercellular mechanisms of genetic exchange. Plasmids are extrachromosomal fragments of DNA containing specific regions or genes encoding functions that enable the replication with different degree of autonomy from the host's replicative proteins and can transfer between bacterial species. Plasmids are classified using typing schemes based on replication or mobilization apparatus (replicon or mobility typing). Generally related plasmids that share the same replicon cannot stably coexist in a cell together and are defined incompatible. Incompatibility (Inc) involves the competition of plasmids for the same replication control machinery within the cell. Competition for replication factors also lead to competition between plasmids (Partridge *et al*., 2018; Rozwandowicz *et al*., 2018).

Intercellular transfer of ARGs via MGEs may play important roles in enhancing the frequency of gene exchange in environments such as farms, hospitals, and sewage systems, which provide ideal incubation conditions for resistance genes acquisition. (Paritridge *et al*., 2018; Davies and Davies, 2010)

In Saudi Arabia, few limited review articles have demonstrated the genetic analysis associated to dissemination of the ARGs via MGEs. Considering the crucial implication of these MGEs in the spread of ARGs across many

bacterial species when they evolve separately from their microbial hosts, this report aimed to give an insight into the epidemiological evolution of antimicrobial resistance by the examination of MGEs in different environments.

## MATERIALS AND METHODS

PubMed and Medline database were used to search relevant literature from 2004 to 2020 using a combination of the following key terms: "antimicrobial resistance," "molecular characterization", "antibiotic resistance genes", "mobile genetic elements", "Saudi Arabia", "genetic analysis". Available peer-reviewed international publications met to the subject of this report were screened for relevance (Table 1). In addition, data of interest from Central for Diseases Control and Prevention and those predicted by the WHO were also included. Only articles written in English are included in this report.

## RESULTS

### Cephalosporins and penicillins resistance

The prevalence of extended-spectrum beta-lactamase (ESBL) producing-gram negative bacilli (GNB) is in augmentation during these last decades, due to the extensive use of the extended-spectrum cephalosporins and penicillin's and their negligible toxicity (Aslam *et al*., 2018). In Saudi Arabia, molecular characterization studies demonstrated the presence of the major ESBL genes (*bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{OXA1}}$, and *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{VEB}}$, *bla*$_{\text{GES}}$, *bla*$_{\text{PER}}$) and CMY AmpC genes ( Alqasim, 2020; Dandachi *et al*., 2019 ; Al-Agamy *et al*., 2014; Al-Agamy *et al*., 2016; Alyamani *et al*., 2017; Leangapichart *et al*., 2016; Al-Hamad *et al*., 2020; Ibrahim *et al*., 2019).

Tawfik *et al*. (2011) demonstrated the existence of *ISEcp1* insertion element linked to *bla*$_{\text{CTX-M-15}}$ genes in 60% of Kp isolates. In this study, all *bla*$_{\text{CTX-M}}$ genes were transferable by conjugation suggesting that genetic structures of all *bla*$_{\text{CTX-M}}$ genes-containing elements are carried by conjugative plasmids. Recently, new study gives more details about the mobility of ESBL genes and showed that CTX-M15 and CTX-M14 ESBL producing-Kp isolates were found to be carried by the conjugative IncFIIK plasmid replicon. The isolates were obtained from patients attending the Microbiology Section of the King Abdulaziz Medical City, Riyadh, Saudi Arabia, between January 2011 and December 2012 (Zaman *et al*., 2018). Contrariwise, diverse plasmids replicons with predominance of IncFIB followed by IncFII and IncFIA have been reported in CTX-M-15-producing clinical *E. coli* isolates that belonged to different ST. The isolates were obtained from King Abdulaziz university hospital of Jeddah (the north west of Saudi Arabia) and collected during 2014-2015. The distribution of ISs in the genomes of CTXM *E. coli* isolates revealed diversity of ISEc with the increase frequency of IS66 and IS21 families, followed by Tn23. (Yasir *et al*., 2020). Furthermore, recent study performed in Jeddah revealed the presence of *ISAba1* element located in the promotor region of *bla*$_{ADC}$ type cephalosporinase gene among carbapenem-resistant *Acinetobacter baumanii* isolates (Shah *et al*., 2019).

### Carbapenem resistance

The genetic analysis of carbapenem resistance-producing MDR bacteria has been well studied (Table 1) and several reports investigated that *bla*$_{\text{OXA}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$ genes carried carbapenem resistance. (Dandachi *et al*., 2019; Al Otaib , 2019; Shah *et al*., 2019; Ibrahim, 2019).. IS903 insertion element has been detected in OXA-48-producing-Kp isolates (Balkhy *et al*., 2012). Moreover, recent studies showed the detection of IS*Aba1* IS upstream of *bla*$_{\text{OXA-23-like}}$ , *bla*$_{\text{OXA-51}}$ like-allele and *bla*$_{\text{OXA-94}}$ genes in multiple carbapenem-resistant isolates of *A. baumannii* from different regions of Saudi Arabia (AL-Agamy *et al*., 2014; Shah *et al*., 2019; AL-Hamad *et al* ., 2020; Al-Amri *et al*., 2020). Indeed, Zaman *et al*. (2018) revealed the presence *ISAba125* flanking *bla*$_{\text{NDM-1}}$ gene in clinical Kp isolates. Also, this gene has been shown be located on IncF conjugative plasmid replicon in *E. coli* isolate from municipal wastewater in Jeddah collected around the Hajj event in October 2013 (Mantilla-Calderon *et al*., 2016). On the other hand, analysis of plasmid typing showed the predominance of IncFIIK plasmid replicon among CTX-M-15 and OXA-48 carbapenamase-co-producing clinical Kp isolates (Zaman *et al*., 2018). The same study demonstrated that *bla*$_{\text{NDM}}$ was found located in combination with *bla*$_{\text{CTX-M-15}}$ on both IncFIA and IncFIB plasmids. As far as *bla*$_{\text{OXA-48}}$, *bla*$_{\text{CTXM-15}}$ and *bla*$_{\text{NDM}}$ genes were found carried together by the conjugative IncL/M plasmid (Zaman *et al*., 2018). In the same line, recent study has been conducted on uropathogenic *E. coli* isolates recovered from urinary tract infections patients in Riyadh between November 2014 and January 2015 and showed the detection of *bla*$_{\text{CTX-M-15}}$, *bla*$_{NDM}$ and *bla*$_{OXA-181}$ in the majority of isolates. Typing of plasmid-mediated resistance genes like beta-lactamases using detection in silico of plasmid Finder and pMLST techniques indicated the existence of four major pMLST types with the predominance of IncF and IncI1 pMLST profiles that carried *bla*$_{\text{CTXM-15}}$,

*bla*$_{TEM-18}$, *bla*$_{CMY-42}$ and *bla*$_{OXA-181}$ genes. IncF pMLST profile has been shown to carry *bla*$_{NDM-1}$, *bla*$_{NDM-5}$, *bla*$_{OXA-9}$ and *bla*$_{CTX-27}$ (Abdel Ghany *et al*., 2018).

**Table 1**. Reported mobile genetic elements (MGEs) related to antibiotic resistance genes (ARGs) from Saudi Arabia.

| MGEs carrying ARGs | ARGS | Bacterial strains | Region | Year of sampling | Reference |
|---|---|---|---|---|---|
| Beta-lactam resistance | | | | | |
| *ISEcp1* on conjugative plasmid | *bla*$_{CTXM-15}$ Cephalosporin resistance | Clinical *K . pneumoniae* | Al Quassim | January to June 2008 | [Tawfik *et al*., 2011] |
| IncFIIk conjugative replicon plasmid | *bla*$_{CTXM-15}$ and *bla*$_{CTXM-14}$ Cephalosporin resistance | Clinical *K . pneumoniae* | Riyadh | between January 2011 and December 2012 | [Zaman *et al*., 2018] |
| IncFIB/IncFII/IncFIA Replicon plasmid ISEc (predominance of IS66 and IS21) | *bla*$_{CTXM}$ Cephalosporin resistance | Clinical *E. coli* | Jeddah | During 2014-2015 | [Yasir *et al*., 2020] |
| *ISAba*1 around the promotor region | *bla*$_{ADC}$ Cephalosporin resistance | Clinical *A. baumanii* | Jeddah | During 2015-2016 | [Shah *et al*., 2019] |
| IS903 around *bla*$_{OXA}$ | *bla*$_{OXA-48}$ Carbapenem resistance | Clinical *K . pneumoniae* | Riyadh | March 2010 | [Balkhy *et al*., 2012] |
| *ISAba125* surround *bla*$_{NDM}$ | *bla*$_{NDM-1}$ Carbapenem resistance | Clinical *K . pneumoniae* | Riyadh | Between January 2011 and December 2012 | [Zaman *et al*., 2018] |
| *ISAba*1 upstream of *bla*$_{OXA}$ | *bla*$_{OXA-23-like\ allele}$ *bla*$_{OXA-51-like\ allele}$ *bla*$_{OXA-94}$ Carbapenem resistance | Clinical *A. baumanii* | Riyadh<br>Quatif<br>Jeddah<br>Dammam | 2011<br>January 2014<br>During 2015 and 2016<br>September 2017 to May 2018 | [Al Agamy *et al*., 2014]<br>[Al Hamad *et al*., 2020]<br>[Shah *et al*., 2019]<br>[Al Amri *et al*., 2020] |
| IncF plasmid | *bla*$_{NDM-1}$ Carbapenem resistance | *E. coli* from municipal wastewater | Jeddah | October 2013 | [Mantilla-Calderon *et al*., 2016] |
| IncFIA/IncFIB replicon plasmid | *bla*$_{CTXM-15}$/*bla*$_{NDM-1}$ Co-harboring Cephalosporin/ carbapenem resistance | Clinical *K . pneumoniae* | Riyadh | Between January 2011 and December 2012 | [Zaman *et al*., 2018] |
| IncL/M conjugative replicon plasmid | *bla*$_{OXA-48}$/*bla*$_{NDM-1}$/ *bla*$_{CTXM-15}$ Co-harboring Carbapenem/cephalosporin resistance | Clinical *K . pneumoniae* | Riyadh | Between January 2011 and December 2012 | [Zaman *et al*., 2018] |
| IncF/IncI1 pMLST | *bla*$_{CTXM-15}$/*bla*$_{TEM-18}$/ *bla*$_{CMY-42}$/ *bla*$_{OXA-181}$ Co-harboring Carbapenem/cephalosporin resistance | Clinical *E. coli* | Riyadh | November 2014 and January 2015 | [Abd El Ghany *et al*., 2020] |
| IncF pMLST | *bla*$_{NDM-1}$/*bla*$_{NDM-5}$ /*bla*$_{OXA-9}$/*bla*$_{CTXM-27}$ Co-harboring Carbapenem/cephalosporin resistance | Clinical *E. coli* | Riyadh | November 2014 and January 2015 | [Abd El Ghany *et al*., 2020] |
| Vancomycin resistance | | | | | |
| New *Tn1546* variants containing *IS1485* and | vanA | *E. faecium* | Riyadh | During 2000-2003 | [Khan *et al*., 2008] |

| | | | | | |
|---|---|---|---|---|---|
| *IS1216V* | | | | | |
| *Tn1546* variants containing *IS1251* and *IS1216V* | vanA | *E. faecium* | Dammam | Between February 2006 and December 2007 | [Khan *et al*., 2013] |
| *Tn1546* variants containing *IS1216V* | vanA | *E. faecium* | Makkah | NM | [Tahir *et al*., 2018] |
| rep11 (repA-pB82) and rep18 (repA-pEF418) plasmid replicons | vanA | *E. faecium* | Makkah | NM | [Tahir *et al*., 2018] |

NM: non-mentioned

Limited data documented the incidence of MGEs like integrons carried carbapenem resistance genes (Table 1). In fact, Al-Agamy *et al*. (2009) detected $bla_{\text{VIM-like}}$ determinants as part of a gene cassette of class 1 integrons in all their MBL-producing *Pseudomonas aeruginosa* isolates from a hospital in Riyadh in 2007. Furthermore Shah *et al.*, (2019) detected the presence of class 1 integrons among carbapenem-resistant *A. baumanii* isolates harboring $bla_{\text{VIM}}$, $bla_{\text{OXA}}$ and $bla_{\text{IMP}}$ genes.

**Table 2**. Results of genetic profile analysis associated with antibiotic resistance genes in Saudi Arabia.

| | **ARGs** | | | |
|---|---|---|---|---|
| **MGEs** | **Cephalosporins** | **Carbapenem** | **Co-harboring carbapenem/ cephalosporin** | **Vancomycin** |
| **Plasmids types** | IncFIIK: $bla_{\text{CTX-M-15, CTX-M-14}}$<br>IncFIB/IncFIA: $bla_{\text{CTX-M}}$ | IncF: $bla_{\text{NDM-1}}$ | IncFIA/IncFIB: $bla_{\text{CTX-M15}}$/ $bla_{\text{NDM-1}}$<br>IncL/M: $bla_{\text{OXA-48}}$/$bla_{\text{NDM-1}}$/ $bla_{\text{CTXM-15}}$<br>IncF/IncI1 : $bla_{\text{CTXM-15}}$/$bla_{\text{TEM-18}}$/ $bla_{\text{CMY-42}}$/$bla_{\text{OXA-181}}$ | Rep11(repA-pB82<br>Rep18(repA-pEF418) |
| **Insertion sequences types** | ISEcp1: $bla_{\text{CTX-M-15}}$<br>ISEc/IS66 and IS21: $bla_{\text{CTX}}$<br>ISAba1: $bla_{\text{ADC}}$ | IS903: $bla_{\text{OXA-48}}$<br>ISAba125: $bla_{\text{NDM-1}}$<br>ISAba1: $bla_{\text{OXA-23-like allele}}$ $bla_{\text{OXA-51-like allele}}$ $bla_{\text{OXA-94}}$ | | IS1485<br>IS1216V<br>IS1251 |
| **Transposon** | | | | Tn1546 |

MGEs: mobile genetic elements; ARGs: antibiotic resistance genes; IS: insertion sequence

**Vancomycin resistance genes**

Vancomycin-resistant *enterococci* (VRE) (VanA, VanB, VanC, VanD, VanE, VanG, VanL, VanM and VanN) phenotypes have been described related to eight types acquired operons (vanA /B / C/ D/ E/ G/ L/ M/ N) confer resistance to vancomycin.

The universal *Tn1546* transposon element was found to carry vancomycin-resistant VanA gene in *E. faecium* clinical isolates obtained from Riyadh during 2000-2003. (Khan *et al*., 2008). In this study Khan *et al*. (2008) reported for the first time in the kingdom the detection of new vanA *Tn1546* variants: two lineages, lineage III *(IS1485* insertion in orf1) and lineage IV (*IS1216V* insertion between vanS and vanH). Later in further study (Khan *et al*., 2013) they indicated the detection of insertion elements *IS1251* and *IS1216V* among vanA VRE isolates obtained from the King Specialist Hospital, Dammam (the eastern region of Saudi Arabia) between Februray 2006 and December 2007. Interestingly, this study reported new *Tn1546* lineage type F3. Recently the same transposon *Tn1546* was detected carrying van(A, X, H, R, S, Y and Z) genes and containing *IS1216V* in clinical isolates recovered from ICU patient in Al-Nour Specialized Hospital in Makkah (Tahir *et al*., 2018). Plasmid typing analysis showed the detection of rep11 (repA-pB82) and rep18 (repA-pEF418) plasmid replicons associated to *Tn1546* variants VanA VRE isolates (Tahir *et al*., 2018).

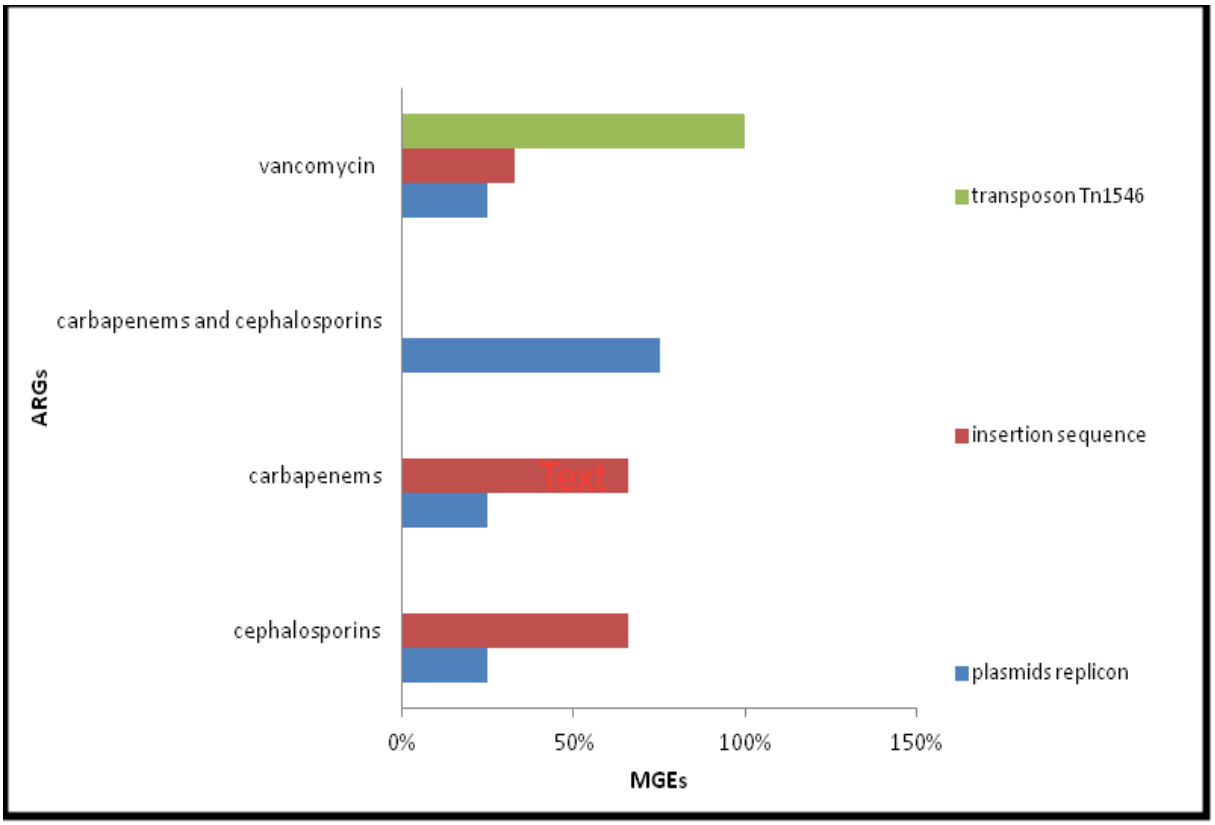


Fig. 1. Percentage distribution of mobile genetic elements (MGEs) (transposon, insertion sequence, plasmids types) carrying antibiotic resistance genes (ARGs) that have been reported in Saudi Arabia.

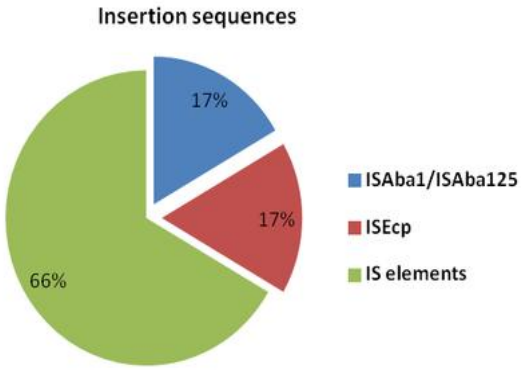


Fig. 2. Distribution of insertion sequences types (ISba1/ISba125, ISEcp, IS elements carrying antibiotic resistance genes (ARGS) (cephalosporin, carbapenem, and vancomycin). Approximately 66% of insertion sequences type IS elements are associated to ARGS.

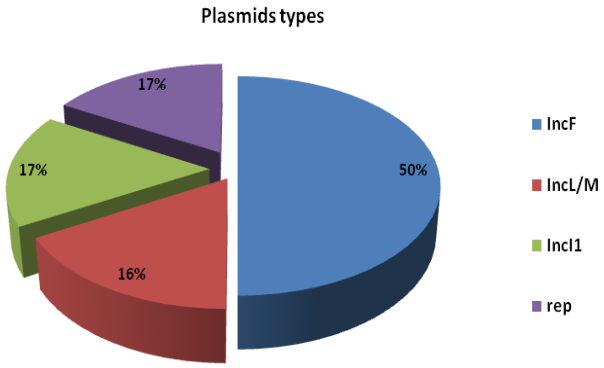


Fig. 3. Distribution of plasmids types (IncF, IncL/M, IncI1 and rep) carrying antibiotic resistance genes (ARGs) (cephalosporin, carbapenem, co-harboring carbapenem/cephalosporin and vancomycin). Approximately 50% of plasmids replicons type IncF are associated to ARGs.

## DISCUSSION

Molecular analysis of ARGs showed high diversity of MGEs as well as clonal diversity (phylogenetic and phylogenomic) in Saudi Arabia (**Fig. 1** and Table 2). In this review it was shown that IS elements played an

important role in the development of antibiotic bacterial resistance. The distribution of insertion sequences (in percentage) carrying cephalosporin, carbapenem, and vancomycin resistance genes showed the predominance of IS elements that represents approximately 66% of IS types followed by ISAba and ISEcp (Table 2 and **Fig. 2**). In fact, the results demonstrated the existence of close linkage between *ISEcp1* and $bla_{\text{CTX-M-15}}$ which are in concordance with several previous publications from many countries of the world including North Africa, Europe and Asia (Mahrouki *et al*., 2012; Lartigue *et al*., 2004). The results obtained confirmed also the importance of IS elements in the plasticity of *E. coli* genome and its evolution and also suggested their role in activating ARGs expression (Siguier *et al*., 2014). Likewise, ISAba IS were found associated with $bla_{\text{OXA}}$ gene. This finding is in agreement with previous publications reported in the golf council countries and worldwide (Dandachi *et al*., 2019; Al Amri *et al*., 2020; Ramirez *et al*.,2020) and therefore highlighted the importance of IS in the epidemic spread of carbapenem resistance genes. Besides, the detection of *IS1251, IS1216V and IS1485* insertion elements showed the diversity of *Tn1546* variants VanA VRE isolates circulating in Saudi Arabian hospitals and incite to provide more attention regarding VRE epidemiology in clinical settings.

Interestingly, basing on genetic profile analysis the distribution of plasmids replicons types (in percentage) carrying cephalosporin, carbapenem, vancomycin and co-harboring of cephalosporin/carbapenem resistance genes showed the predominance of IncF plasmid replicon that represents approximately 50% of plasmid types followed by IncL/M, IncI1 and rep (Table 2 and Fig. 3). The current results in this report highlighted the high incidence of conjugative IncFIIK followed by IncL/M plasmids in CTX-M and carbapenemase-producing isolates. These findings are concomitant with several reports from European, North African and Asian countries (Hamprecht *et al*., 2019; Partridge *et al*., 2018; Mahrouki *et al*., 2015; Dandachi *et al*., 2019) and thus warn the possibility epidemic dissemination of multidrug resistance plasmids worldwide, which constitute an increasing threat for modern medicine. Moreover, the detection of IncF conjugative plasmid replicon carrying $bla_{\text{NDM-1}}$ gene in municipal wastewater (Mantilla-Calderon *et al*., 2016) and then in clinical settings (Zaman *et al*., 2018) highlighted the role of sewage as a major environmental reservoir of AMR that may acts simultaneously as a vector for the dissemination of MGEs.

**Conclusions**

In Saudi Arabia the diversity of MGEs observed may reflect to the extensive population flow from the Middle East and the Indo-Pakistan subcontinent and also to the annual hosting of mass gatherings events (hajj pilgrimage). Furthermore, the poor knowledge regarding antibiotic use among the general population may threaten the continued effectiveness of antimicrobials. Thus, there is an urgent need to specifically address public health intervention, staff training and communication procedures for infection prevention.

**Conflict of interest**

No conflict of interest to declare

**Acknowledgments**

Author would like to acknowledge Northern Borders University, Saudi Arabia for its continuous support to researchers.

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(*Accepted for publication October 2021*)