Doctoral Thesis

Role of Mobile Genetic Elements (MGEs) in the Dissemination of Antimicrobial Resistance Genes in Pathogenic Gram-Negative Bacteria

(Summary)

Ahmed Mahrous Ahmed Soliman

Graduate School of Biosphere Science

Hiroshima University

September, 2020

Antimicrobial resistance (AMR) constitute a major public health threat. AMR is the resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. In the USA, Annual data estimates more than 2.8 million infections with antibiotics-resistant bacteria causing more than 35,000 deaths. Additionally, an estimated 700000 annual deaths occurs worldwide resulting from drug-resistant bacterial infections, malaria, and tuberculosis. Because of drug-resistant infections, an estimated 10 million deaths will be observed by 2050, with economic losses more than US$ 100 trillion. Our world is very close to the situation in the pre-antibiotic era, especially because there are no new antibiotics in the pipeline.

There are two types of bacterial AMR: (i) intrinsic, which results from spontaneous chromosomal mutation, (ii) acquired through exchange of mobile genetic elements (MGE) which includes plasmids, transposons, insertion sequence, integrons, phages, genomic island (i.e., *Salmonella* or *Proteus* genomic island (SGI1/PGI1), integrative and conjugative element (ICE) (i.e., SXT/R391)).

Chapter II describes the role of plasmids, integrons, and insertion sequences (IS) in the dissemination of carbapenemase and colistin resistance genes in pathogenic Gram-negative bacteria as follow:

In study 1, I identified self-transmissible A/C plasmids carrying $bla_{\text{NDM-1}}$ in *E. coli*, *K. pneumoniae*, and *P. stuartii*. Interestingly, $bla_{\text{NDM-1}}$ was surrounded upstream by the IS*Aba125*(either intact or truncated) illustrating the potential for HGT. Gene $bla_{\text{OXA-181}}$

was carried by IncX3 plasmid. Furthermore, I detected an integron-borne *bla*$_{\text{VIM-2}}$, and *bla*$_{\text{VIM-24}}$ in several *P. aeruginosa* clinical isolates from Egypt.

In study 2, I reported the first draft genome sequence of a multidrug-resistant *E. coli* D: ST69 carrying *bla*$_{\text{NDM-1}}$- and *bla*$_{\text{OXA-244}}$. The *bla*$_{\text{OXA-244}}$, and *bla*$_{\text{NDM-1}}$ were located on the chromosome (Tn*6237*) and on an IncI1 type self-conjugative plasmid of >93-kb in size, respectively. Beside the clonal expansion of the *E. coli* ST38 pandemic clone, I further identified that the spread of OXA-244-*E. coli* could be related to the mobilization of the IS*1R*-made composite transposon (Tn*6237*) carrying *bla*$_{\text{OXA-244}}$.

In study 3, two *E. coli* strains were detected to harbor *mcr-1,* showed multidrug-resistant phenotypes, and belonged to phylogroup D: ST744 and phylogroup A: ST1011, respectively. *mcr-1* was located on a conjugable IncFIB (~93 Kb) and a conjugable IncX4 (~24 Kb) plasmids.

In study 4: I described the first full genome sequence of an *mcr-9* and *bla*$_{\text{VIM-4}}$-carrying multidrug-resistant *Enterobacter hormaechei* clinical isolate from Egypt. *mcr-9* and *bla*$_{\text{VIM-4}}$ were carried by a self-transmissible IncHI2 plasmid, pAMS-38a (281,121 bp in size). The genetic context of *mcr-9* and *bla*$_{\text{VIM-4}}$ was identified as IS*1-mcr-9*-IS*903-pcoS*-Δ*pcoE-rcnA* and *intI1-bla*$_{\text{VIM-4}}$*-aac(6')-II-dfrA1*-Δ*aadA23-smr*-IS*Pa21-qacE*Δ*1*, respectively.

In study 5, I reported the first identification of a multidrug-resistant *Klebsiella pneumoniae* ST30 isolate from Japan co-harboring *mcr-9*-, *bla*$_{\text{NDM-1}}$- and *bla*$_{\text{VIM-1}}$. Genes

mcr-9 and *bla*$_{\text{VIM-1}}$ were located on a self-transmissible IncHI2 plasmid (281,251 bp in size) while *bla*$_{\text{NDM-1}}$ was located on a conjugable IncFII(K) plasmid (124,214 bp in size).

Chapter III describes the role of *Salmonella* genomic island 1 (SGI1) in the dissemination of antimicrobial resistance genes in pathogenic Gram-negative bacteria as follow:

In study 6, I reported the first identification of SGI1-positive *P. mirabilis* isolated from chicken flocks in Africa. Additionally, I identified clonally related *P. mirabilis* strains carrying SGI1 and isolated from humans and chicken flocks in Egypt. Here, I clarified the geographical and biological spreading of SGI1 through an inter-transmission pathway.

In study 7, I report the first identification of *Salmonella* genomic island 1 (SGI1) in a multidrug-resistant clinical isolate of *Providencia stuartii*. The strain was detected to carry SGI1 variant SGI1-W with an MDR region of *intI1-aadA2-lnuF-qacEΔ1-sul1*-orf5-orf6-IS*6100*, conferring resistance to five dissimilar antimicrobials including spectinomycin, streptomycin, lincosamides, quaternary ammonium compounds, and sulfonamides. These data raise concerns about the possible dissemination of SGI1 in another genus of the Enterobacteriaceae.

In study 8, I report the emergence of a SGI1-harboring multidrug resistant *K. pneumoniae* human clinical isolate from Egypt; this strain carried SGI1 variant SGI1-C. The presence of SGI1 in *K. pneumoniae,* one of the most commonly isolated species of Enterobacterales, is very important as it identify another mechanism of spreading and

transfer of antimicrobial resistance and virulence genes. Implementation of screening strategies for SGI1 and other SGI1-related elements among Gram-negative bacteria (not only *Salmonella* spp. or *P. mirabilis*) are essential to prevent the spreading of this element to other human pathogen.

In conclusion, I illustrated experimentally the crucial role of different MGEs in the dissemination and spread of pathogenic and antimicrobial-resistant Gram-negative bacteria. From One Health and Global Health perspectives, I isolated several multidrug resistant bacterial strains from different ecological niches (i.e., hospitals, poultry farm, and chicken meat) from two different countries (i.e. Egypt and Japan). Additionally, development of novel antimicrobial agent or novel alternatives for antimicrobials (i.e., phage therapy and endolysins) is extremely important to prevent the entrance to the pre-antibiotic era. Furthermore, development of novel rapid, easy to perform, and cost-ineffective diagnostic tests for detection of such bacteria is pivotal to prevent its spreading. This study further confirmed the urgent need for active screening policies for such MGEs, antimicrobial surveillance plans, and infection control measures to successfully and efficiently stop the spread of such superbugs.