Appendix A

MGEs & AMR dissemination

MGEs promote intercellular and intracellular DNA mobility. The intercellular mobility of DNA represents its mobility between bacterial cells by three mechanisms: conjugation, transformation, and transduction [1]. Conjugation is the transfer of DNA via pili structures between two adjacent bacteria [1] through replicating genetic elements: conjugative plasmids or the chromosomally Integrated Conjugative Elements (ICEs) (e.g. conjugative transposons (cTns). In contrast, transformation depends on the uptake of free DNA by competent cells[1,2]. Also, transduction is mediated by replicating bacterial viruses (called bacteriophages) that transfer bacterial DNA between a bacteriophage-infected and a bacteriophage-susceptible bacteria [1]. On the other hand, intracellular DNA mobility is mediated by integrons and transposable elements (transposons and insertion sequences) that can move (transpose) from one site in the genome to a second site, or from one DNA molecule (that is an infecting phage genome or a plasmid) to a second DNA molecule (the bacterial chromosome) [3]. Interestingly, they also could be transferred to other cells through plasmids or phages[2].

Plasmids

Plasmids are extrachromosomal DNA capable of autonomous replication. They harbour ARGs that confer resistance to various antimicrobial agents. They are considered a corner stone in horizontal gene transfer (HGT) among bacteria, which significantly results in the dissemination of ARGs among different species and genera of bacteria [4]. Plasmids are classified into incompatibility groups depending on their stability during conjugation [5]. Just as bacteria is typed into clones, plasmid typing help epidemiologists know the “kind” or “type” of plasmids carrying same or different ARGs. Through plasmid typing, the presence of a single or multiple plasmids carrying or mediating the spread of ARGs between bacteria can be easily identified to inform epidemiological interventions [6,7]. For instance, IncF

plasmids are known to be promiscuous with a narrow host range i.e., they can carry multiple ARGs and can be hosted by selected bacterial species, facilitating the spread of ARGs into different bacterial species. Further, the presence of *bla*$_{NDM}$ on IncX or IncH plasmids in several countries and species shows that these particular plasmid replicons are facilitating the spread of *bla*$_{NDM}$ across different species and through them, into different countries[6,7].

Integrons

A gene cassette is a small mobile element that consists of a single gene (lacks a promoter) and an *attC* recombination site. A gene cassette could be found inserted into an integron, which consists of an *intI* gene, an *attI* recombination site, and a promoter (Pc). *IntI* encodes a site-specific recombinase that catalyzes recombination between the *attI* site of the integron and the *attC* site of a gene cassette [8]. Integrons are found mostly on bacterial chromosomes and plasmids, and have a role in generating diversity in bacterial genomes, plasmids, and transposons as well as in sharing information among bacteria [9]. There are different classes of integrons depending on the sequence of *intI* (for example *intI1*, *intI2*, i*ntI3*, etc.). Class 1 integrons are commonly associated with AMR in clinical isolates [8,10].

Transposable elements

Transposable sequences, which include insertion sequences (ISs) and Transposons (Tn), are discrete DNA segments (short sequences) capable of integrating themselves (with the associated ARGs) into new locations on the same or different plasmids/chromosomes within a single cell [8]. Many ISs include a promotor that participate in the expression of the ARGs. Also, the upstream location of ISs to intrinsic chromosomal ARGs influences resistance; in *Acinetobacter baumannii,* the upstream location of IS*Aba1* by *bla*$_{OXA\text{-}51\text{-}like}$'s promoter increases resistance to carbapenems [11].

Appendix B

Currently, multi-locus sequence typing (MLST), which involves the use of at least seven house-keeping genes specific to each bacterial species, is used to identify the species as well as distinguish between the different strains/clones within that species [12]. MLST has international appeal as it enables scientists from different laboratories worldwide to compare their results easily, making MLST better than PFGE (pulsed-field gel electrophoresis). PFGE is another typing tool that is gradually being faded away as it does not allow easy cross-laboratory comparison of bacterial typing data. Finally, PFGE is very laborious and time-consuming, making MLST an easier and more acceptable [13]. However, both PFGE and MLST are also giving way to whole-genome-based bacterial typing in which the core genome of the whole genome is used to 'type' and classify bacteria into clades, sub-clades, and clones [14]. Core-genome whole-genome typing (cg-WGT) or core-genome MLST (cg-MLST) thus involves the use of several thousands of core genes instead of just seven or eight house-keeping genes. Thus, cg-MLST provides a higher resolution and more robust typing system than MLST [15,16].

Further, the sequence type of any bacterial species can be obtained from the whole genome, giving whole-genome sequencing an edge over MLST. Indeed, species that have been classified into the same sequence type by MLST have been shown by cg-MLST to be rather distant from each other [17]. Hence, phylogenetic trees made from whole genomes are preferable to MLST dendrograms for tracing bacterial infections. cg-MLST is not the only method used to type bacteria using whole genomes. Other methods also type bacteria using all the genomes and not only the core genome. In such methods, the whole genomes of the bacteria are aligned, and single nucleotide polymorphisms (SNPs) are detected, which are then used to draw a phylogeny tree. A cut-off SNPs number is normally then used to assign

the isolates into clades, sub-clades, and clones. Thus, non-cgMLST typing methods involve more genes (as well as variable sections of the genome) than cg-MLST [18,19].

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