SATURDAY - AAR-620

Impaired DNA Mismatch Repair System Increases the Risk of Emerging Antibiotic Resistance in Bacteria

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Background

DNA mismatch repair system (MMR) is an important mutation surveillance system for recognizing and repairing errors that occur in the newly synthesized strand during DNA replication and mismatches generated during genetic recombination. We hypothesized that an impaired MMR system will increase the mutation rate in bacteria, increasing the mutation within genes conferring resistance to antibiotics such as fluoroquinolone resistance related mutations (Ser83 and Asp87 in gyrase A, Ser87 in ParC) and carbapenem resistance related mutations (porin and efflux system). In this study, we investigated the effect of loss of function (LOF) mutations in the MMR system on the emergence of fluoroquinolone resistance related mutations in bacteria.

Methods and Materials

Whole genome sequences of clinical isolates from NCBI:

- 1331 *Pseudomonas aeruginosa*
- 6425 *Escherichia coli*
- 5026 *Klebsiella pneumoniae*

OpGen Acuitas® Whole Genome Sequencing pipelines.

Results

Mismatch Repair System in Gram-negative Bacterium

The mismatch repair system corrects the errors which occur during the DNA replication process, and ensure the genetic information is transferred faithfully. It has been found in most organisms: from bacteria to human. **Table 1** lists the *E. coli* genes involved in the mismatch repair system. Among these genes, mutS , mutL and mutH are specific to the mismatch repair function. Others also function in other processes. The reference sequence used for these genes are listed in **Table 2.**

Table 1. *E. coli* Mismatch Repair System

Gene	Function
MutS	Mismatch recognition
MutL	Signal propagation, recuited by MutS after mismatch is detected
MutH	Strand discrimination, recruited by MutS, MutL complex, nicking new synthesized strand
UvrD	Unwinding from the nick created by MutH
DNA exonuclease	Degrade the new synthesized strand with mismatch from the nick to at lease the mismatched base
DNA polymerase	Filling the gap
DNA ligase	Filling the nick
Dam methylase	Methylation of newly synthesized strand

Table 2. Mismatch Repair System Specific Genes in *E. coli, K. pneumoniae* and *P. aeruginosa*

Organism	Gene Symbol	NCBI Accession
E. coli	MutS	NC_000913.3:2857093-2859654
	MutL	NC_000913.3:4397412-4399259
	MutH	NC_000913.3:2969662-2970351
K. pneumoniae	KPHS_41690	NC_016845.1:4198814-4201375
	KPHS_04140	NC_016845.1:452067-453614
	KPHS_43040	NC_016845.1:4339801-4340496
P. aeruginosa	MutS	NC_002516.2:4054525-4057092
	MutL	NC_002516.2:c5551681-5549780

Effect of MMR mutation on the antibiotic resistance related mutation rate

Table 3. Number of isolates analyzed for mutation in mismatch repair system

Species	Number with MMR mutation	Number without MMR mutation	Total
P. aeruginosa	59	1272	1331
E. coli	38	6387	6425
K. pneumoniae	29	4997	5026

While 4.4% of analyzed *P. aeruginosa* isolates carry LOF mutation in MMR specific genes, only 0.59% of analyzed *E. coli* and 0.58% of analyzed *K. pneumoniae* isolates carry these mutations (**Table 3**), suggesting the antibiotic resistance-related mutation is more prominent in *P. aeruginosa*.

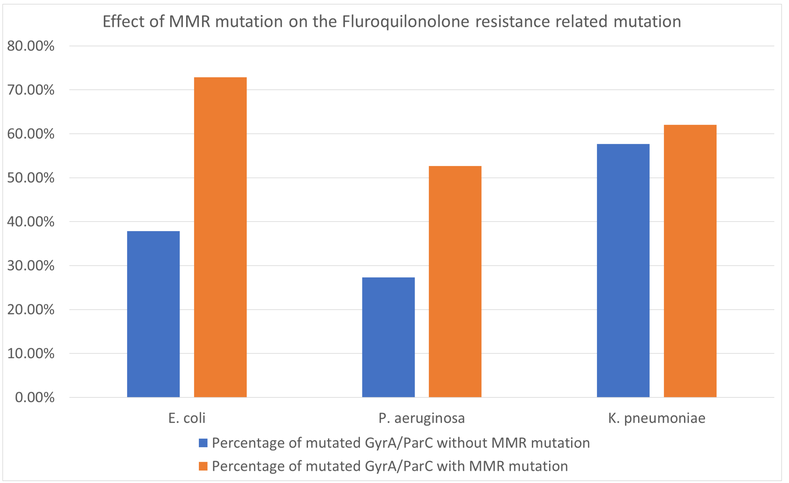

Figure 1. Effect of MMR mutation on the fluoroquinolone resistance related mutation rate

Higher proportions of *E. coli* and *P. aeruginosa* isolates with LOF mutation in MMR specific genes carry the fluoroquinolone resistance related mutation (**Table 4** and **Figure 1**). We observed less significant change in *K. pneumoniae* isolates. However, this might be result of the fact that the higher proportion of analyzed *K. pneumoniae* isolate have fluoroquinolone resistance related mutation: 57.7% compared with 39.4% *for P. aeruginosa* and 27.4% for *E. coli*.

Table 4. Higher proportion of gram-negative bacteria with Impaired MMR system carry antibiotic resistance-related mutations

	Number with Gyrase/ParC Mutation	Number without Gyrase/ParC Mutation	Percentage with GyrA/ParC Mutation
P. aeruginosa			
Isolates with LOF mutation in MMR	43	16	72.9
Isolates without LOF mutation in MMR	481	807	37.3
E. coli			
Isolates with LOF mutation in MMR	20	18	52.6
Isolates without LOF mutation in MMR	1745	4642	27.3
K. pneumoniae			
Isolates with LOF mutation in MMR	18	11	62.1
Isolates without LOF mutation in MMR	2883	2114	57.7

Conclusion

- We demonstrated that higher proportions of isolates with LOF mutations in MMR genes carry fluoroquinolone resistance-related mutations, suggesting that an LOF mutation in the MMR system genes will increase the mutation rate, leading to an increase in the risk of emerging antibiotic resistance in bacteria.
- Among analyzed isolates, higher proportion of *P. aeruginosa* carry the LOF mutation in MMR specific genes, suggesting that the antibiotic resistance-related mutation is more prominent in *P. aeruginosa.*