Increased Use of Carbapenems Can Lead to Highly Resistant Bacteria

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In May 2005 a major outbreak caused by a single *Klebsiella pneumoniae* strain occurred at Uppsala University Hospital, Sweden. The bacterial strain spread through the hospital and affected close to 300 patients. It produced enzymes that could degrade a lot of commonly used antibiotics, thereby making the bacterium tolerant, or resistant, to these antibiotics. One of these enzymes is called extended spectrum β-lactamase (ESBL). Disease causing bacteria that can produce these enzymes are a major threat in clinical settings because they make treatment of a disease very difficult. There are circular DNA elements called plasmids present in bacteria that often carry such antibiotic resistance genes. The plasmid found in the strain that caused the outbreak had a gene for an ESBL that has been reported worldwide. It is called the CTX-M-15 enzyme. Carbapenems are a relatively new class of antibiotics that has been found to be effective against ESBL-producing bacteria. Even though there were no carbapenem-degrading enzymes on the plasmids the strain carrying it showed increased tolerance to carbapenems. For this study two types of carbapenems: ertapenem and meropenem were used. The bacterium *Escherichia coli* (*E. coli*) was used to study how a strain carrying the outbreak plasmid would change in response to use of carbapenem drugs. *E. coli* was chosen because it is a common pathogen and there have been few reports of carbapenem resistance associated with it.

In *E. coli* there are protein channels (porins) that allow uptake of nutrients. Antibiotics often enter through those channels as well. In a previous study when *E. coli* with the plasmid from outbreak was grown in presence of meropenem and ertapenem it was found that the bacteria responded by changing and losing these porins. In order to study other mechanisms of resistance I used an *E. coli* strain from which these proteins were already deleted. The results showed that the most common resistance mechanism in a porin deleted strain was increasing the number of its ESBL-genes. So when in the beginning there was one gene producing ESBL-enzyme after increase there were more than 90 copies producing it. This increased the resistance of the strain to meropenem 48-folds, which is extremely high.

For the bacteria there is however, one disadvantage of so many copies of a gene. It grows much slower as compared to its original one copy strain. In the environment the strain that grows faster will always be at an advantage. The only situation where the strain with increased copies would be at an advantage is when there is a high concentration of meropenem present, and then it may be the only strain that survives.

I also determined how a strain without this outbreak plasmid would respond to carbapenem use. It was seen that in order to survive high concentration of meropenem several mutations accumulated in its DNA and they combined to give it a high resistance to meropenem.

In conclusion my data suggests that carbapenem resistance will arise in ESBL-producing bacteria without porins by increase in the copy number of β-lactamase genes but the resistant bacteria would be less fit and unable to survive. Carbapenem use however needs to be monitored because the data also suggests that in the presence of high concentration of meropenem very high resistance levels can be achieved.

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